Journal Club

Editor's Note: These short, critical reviews of recent papers in the *Journal*, written exclusively by graduate students or postdoctoral fellows, are intended to summarize the important findings of the paper and provide additional insight and commentary. For more information on the format and purpose of the Journal Club, please see http://www.jneurosci.org/misc/ifa_features.shtml.

Change of AMPA Receptors in Spinal Nociceptive Synapses during Inflammatory Hyperalgesia

Wen Wang[1,2,3]* **and Jing Huang**[1]*

[1]Department of Anatomy and K. K. Leung Brain Research Centre, Fourth Military Medical University, [2]Department of Psychosomatics, Institute of Clinic Neuroscience, Xi'jing Hospital, Fourth Military Medical University, Xi'an 710032, People's Republic of China, and [3]Division of Cerebral Structure, National Institute for Physiological Sciences, Okazaki 444-8787, Japan

Review of Larsson and Broman (http://www.jneurosci.org/cgi/content/full/28/28/7084)

Nociceptive primary afferent fibers, which convey noxious information from the periphery to the spinal dorsal horn, are commonly divided into those that express neuropeptides such as substance P and calcitonin gene-related peptide (CGRP), and those that show little or no expression of neuropeptides. Peptidergic nociceptive afferent fibers synapse onto projection neurons in lamina I, most of which express neurokinin 1 receptors (NK1Rs). In contrast, nonpeptidergic nociceptive afferent fibers project to lamina II and synapses mainly onto intrinsic inhibitory and excitatory interneurons.

Central sensitization of spinal noxious synapses contributes to inflammatory hyperalgesia via long-term potentiation (LTP) of noxious glutamate neurotransmission. LTP can occur in either lamina I or II with different consequences. LTP of lamina I projection neurons can amplify the noxious information from the periphery, which might contribute directly to the observed hyperalgesia. In contrast, LTP of lamina II neurons can produce either amplification or inhibition of noxious transmission, depending on the excitatory or inhibitory nature of these intrinsic neurons as well as their targets (for example, primary afferent fibers, projection neurons, descending terminals, etc.).

Like LTP at some forebrain synapses, it is assumed that translocation of AMPA receptors (AMPARs) containing the GluR1 subunit to the noxious synapses is a key event in the initial phase of LTP that underlies acute inflammatory hyperalgesia. This translocation of GluR1-containing AMPARs requires activation of CaMKII. Based on the above-mentioned assumption, there should be increased GluR1 insertion into postsynaptic membrane for both lamina I and II noxious synapses, accompanied by increased activation of CMKII in the corresponding regions, during acute inflammatory hyperalgesia.

Larsson and Broman (2006) provided data that are partly consistent with this hypothesis. The nonpeptidergic primary afferent fibers were labeled by transganglionically transported *wheat-germ* agglutinin-horseradish peroxidase (WGA-HRP) injected into one hindpaw of rat. Three days later, capsaicin was injected into the same region to induce inflammatory hyperalgesia. Twenty minutes after capsaicin injection, the rats were killed and the ultrastructural localization of GluRs and CaMKII and pCaMKII (the active, phosphorylated form of CaMKII) was examined using electron microscopy after postembedding immunogold labeling. The authors showed that capsaicin-induced acute inflammatory hyperalgesia was accompanied by increased levels for both CaMKII and pCaMKII in lamina I noxious synapses, but decreased levels for both CaMKII and pCaMKII in lamina II noxious synapses.

In accordance with this, one would expect to find increased insertion of GluR1 in lamina I peptidergic noxious synapses and possibly decreased insertion in lamina II nonpeptidergic noxious synapses in capsaicin-induced acute inflammatory hyperalgesia. In contrast to this expectation, however, Larsson and Broman (2008) showed that capsaicin-induced acute inflammatory hyperalgesia is accompanied by increased postsynaptic density of GluR1 in lamina II nonpeptidergic noxious synapses and unchanged postsynaptic density of GluR1 in lamina I peptidergic noxious synapses. The postsynaptic density of GluR2/3 in lamina II nonpeptidergic and lamina I peptidergic noxious synapses did not change during capsaicin-induced hyperalgesia. Furthermore, hyperalgesia was not accompanied by change of noxious synapse

Received Aug. 21, 2008; revised Sept. 3, 2008; accepted Sept. 3, 2008.

This work was supported by National Natural Science Foundation of China Grants 30500278 and 30771133, Chinese Military "Eleventh Five-year project" Grant 06H037, Innovation Research Team Program of the Ministry of Education of China Grant IRT0560, and National Program of Basic Research of China Grant G2006CB500808. We thank Profs. Yun-Qing Li, Sheng-Xi Wu (Fourth Military Medical University), and Ryuichi Shigemoto (National Institute for Physiological Sciences of Japan) for insightful comments on this article.

*W.W. and J.H. contributed equally to this work.

Correspondence should be addressed to Dr. Wen Wang, Department of Anatomy and K. K. Leung Brain Research Centre, Fourth Military Medical University, Xi'an 710032, People's Republic of China. E-mail: wangwen@fmmu.edu.cn.

DOI:10.1523/JNEUROSCI.4011-08.2008

 0270-6474/08/2810185-02$15.00/0

size. These findings may suggest that LTP exists in lamina II but not in lamina I noxious synapse, and raise two questions. First, is it true that LTP does not occur at lamina I noxious symapses? Second, why is increased activation of CaMKII in lamina I not accompanied by increased GluR1 insertion?

Low-frequency (2 Hz) stimulation (LFS), which resembles the continuous low-frequency afferent (C-fiber) barrage that occurs during inflammation, can induce LTP in lamina I noxious synapses (Ikeda et al., 2006). Meanwhile, *in vivo* observation confirmed that lamina I NK1R-positive projection neurons are important for capsaicin-induced acute inflammatory hyperalgesia (Mantyh et al., 1997). Thus, the answer to the first question is likely that lamina I NK1R-positive projection neurons can establish LTP during capsaicin-induced inflammatory hyperalgesia, but there must be some other mechanisms underlying this form of LTP. However, it is difficult to answer the second question based on current data.

Larsson and Broman (2008) gave several possible answers to the two aforementioned questions, including the following. (1) Other changes such as phosphorylation of already inserted GluR1 contribute to the observed LTP of lamina I noxious synapses. (2) Activation of synaptic CaMKII might not be sufficient to translocate GluR1 to synapse. (3) There are slower translocations of GluR1 to the synapses in lamina I than in lamina II. (4) LTP of lamina I noxious synapses contributes to the latter phase of inflammatory hyperalgesia. Based on our reading on this study and other recent studies, we suggest another possibility.

Ikeda et al. (2006) performed whole-cell recording on lamina I projection neurons identified by retrograde labeling with a fluorescent tracer, DiI (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate), injected into either parabrachial nuclei (PBN) or periaqueductal gray (PAG). LFS was given to assess LTP in the lamina I neurons. The authors found that LFS did not cause any LTP in any of the tested lamina I neurons that projected to the PBN (spino-PBN neuron), but LFS did cause LTP in all of the tested lamina I neurons that projected to the PAG (spino-PAG neuron). According to a previous report (Spike et al., 2003), >90% of PAG-projection neurons in lamina I (in their study, total number of PAG-projection neurons in lamina I is 120 of 400 projection neurons) also sent axons to PBN. Assuming that Ikeda et al. (2006) only recorded neurons that project purely to the PAG, the number of projection neurons that display LTP would be <12, or <3% of lamina I projection neurons. Although consistently recording from such a small number of neurons would be difficult, suggesting at least some of the spino-PAG neurons that Ikeda et al. examined also projected to PBN, making it difficult to assess the proportion of lamina I projection neurons that are potentiated by LFS. Nevertheless, even if LTP can be induced by LFS in all spino-PAG neurons and only in these neurons, and assuming a homogeneous peptidergic innervation of lamina I projection neurons, ~30% of peptidergic synapses would be potentiated, and it is possible that the increased levels of GluR1 would not be detectable. In fact, Larsson and Broman did show a slight but not quite statistically significant increase in postsynaptic GluR1 levels at peptidergic synapses.

The question remains why there is such a prominent increase in pCaMKII and CaMKII in lamina I synapses. Do other changes, such as phosphorylation of already inserted GluR1, contribute to the observed LTP at lamina I noxious synapses? Is activation of synaptic CaMKII insufficient to translocate GluR1 to synapse? We are waiting for the further experimental evidence.

Larsson and Broman provided a very useful protocol: anterograde labeling nonpeptidergic primary nociceptive afferent fibers combined with postembedding immunogold labeling of AMPAR subunits and related molecules. One of its advantages is that the synapses receiving nociceptive information via nonpeptidergic fibers can be identified, thus offering informative measurements such as size of noxious synapses and membrane density of certain molecules. It can be applied in other pain models (acute or chronic) to investigate the underlying central sensitization, focusing on the nonpeptidergic fibers' contribution.

In conclusion, Larsson and Broman reported the phenomenon of unchanged insertion of GluR1 to lamina I noxious synapses and increased insertion of GluR1 to lamina II noxious synapses during capsaicin-induced acute inflammatory hyperalgesia. In addition to those discussed by Larsson and Broman, we offer an additional possible explanation for the observed lack of capsaicin-induced recruitment of GluR1 to peptidergic synapses: The increased insertion of GluR1 to lamina I noxious synapses is diluted by the dominant population of projection neurons that do not show LTP during hyperalgesia. The protocol used in their study has the potential to be applied in other pain models to elucidate the underlying mechanisms for central sensitization.

References

Ikeda H, Stark J, Fischer H, Wagner M, Drdla R, Jäger T, Sandkühler J (2006) Synaptic amplifier of inflammatory pain in the spinal dorsal horn. Science 312:1659–1662.

Larsson M, Broman J (2006) Pathway-specific bidirectional regulation of Ca^{2+}/calmodulin-dependent protein kinase II at spinal nociceptive synapses after acute noxious stimulation. J Neurosci 26:4198–4205.

Larsson M, Broman J (2008) Translocation of GluR1-containing AMPA receptors to a spinal nociceptive synapse during acute noxious stimulation. J Neurosci 28:7084–7090.

Mantyh PW, Rogers SD, Honore P, Allen BJ, Ghilardi JR, Li J, Daughters RS, Lappi DA, Wiley RG, Simone DA (1997) Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. Science 278:275–279.

Spike RC, Puskár Z, Andrew D, Todd AJ (2003) A quantitative and morphological study of projection neurons in lamina I of the rat lumbar spinal cord. Eur J Neurosci 18:2433–2448.