Archives Italiennes de Biologie, 143: 103-112, 2005.

MODULATION OF SENSORY INPUT TO THE SPINAL CORD BY PRESYNAPTIC IONOTROPIC GLUTAMATE RECEPTORS

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The centrally directed process of dorsal root ganglion (DRG) neurons conveys peripheral stimuli to the spinal cord and dorsal column nuclei. From these stations, other central neurons, in thalamus and/or brainstem, are interposed before the peripheral event finally reaches the somatosensory cortex. The perception of quality and intensity of the stimulation is not linearly related to the physical properties of the stimulus itself as a result of modulatory mechanisms present at all levels of the chain of propagation of the peripherally-generated signal.

The unique functional characteristics of synaptic endings at different levels of the somatosensory path contribute to the modulation of the release of their neurotransmitter and, thus, to their synaptic efficacy. The release of neurotransmitter by terminals of the central process of DRG neurons is modulated by a variety of presynaptic mechanisms. As an example of these mechanisms, DRG neurons express and transport receptors that are inserted in the membrane of the central axon terminal and are, therefore, referred to as presynaptic receptors. These include ligand-gated ionotropic and metabotropic receptors. Receptor ligands include the main inhibitory neurotransmitter in the CNS, γ-aminobutyric acid (GABA). Receptors for GABA can be the ionotropic subtype A, $GABA_A$ (3) and the metabotropic subtype B, $GABA_B$ (76). Presynaptic GABA receptors on central terminals of DRG neurons are believed to play an important role in one of the best studied forms of modulation of peripheral input to the spinal cord, presynaptic inhibition. This form of inhibition is mediated by axo-axonic contacts by GABAergic interneurons, and was reported to affect large myelinated, low-threshold mechanosensory (Aα) fibers (2, 69). Terminals of these interneurons activate $GABA_A$ receptors at the synaptic endings of DRG afferents thereby opening Cl^- channels (69). The equilibrium potential for Cl^- in DRG central terminals is above resting potential, due to the expression in DRG neurons of a Cl^- co-transporter which uses the energy from the Na^+ gradient generated by the Na^+-K^+-ATPase pump to increase the intracellular concentration of Cl^-. Thus, the opening of Cl^- channels in DRG terminals results in an efflux of Cl^- ions which depolarizes, rather than hyperpolarizes, the axon terminal membrane (4, 81). Depolarization of the synaptic ending can inhibit release of neurotransmitter by

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blocking propagation of action potential via inactivation of voltage-gated Na^+ channels, or by an increase in membrane conductance that "shunts" Na^+ currents (26, 27, 30). Evidence for presynaptic inhibition of small myelinated (Aδ) and unmyelinated (C) fibers is not as robust as for Aα fibers, but several investigators have suggested depolarization of terminals of these afferents (14, 18, 28, 32, 66). However, the sparseness of GABAergic axo-axonic contacts over terminals of C fibers (2, 11) and the absence of dorsal root reflexes in C fibers (81) suggest that mechanisms other than classic presynaptic inhibition may be responsible for the modulation of C fiber afferents in spinal cord (69).

Besides GABA receptors, DRG neurons express both ionotropic and metabotropic glutamate receptors (20, 33, 44, 45, 49, 52, 59, 62, 63, 71, 74). Both types of glutamate receptors are transported peripherally (15, 22), and centrally (16, 35, 37, 42, 43, 45, 47, 48). Peripherally transported receptors can be activated by application of glutamate or its agonists to the skin (15, 77, 83). Their discovery has opened the way for a new interpretation of the mechanism for sensitization and for the exploration of novel therapeutic strategies for pain (23, 25). The remainder of this article will focus on presynaptic ionotropic glutamate receptors (PIGR) in terminals of DRG central processes and their functional properties.

Different ionotropic glutamate receptors are identified, and referred to, according to the agonist that activates them: i) kainate, ii) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and iii) *N*-methyl-D-aspartic acid (NMDA). Receptors in each group can be heteromeric association of different subunits. PIGR in central terminals of DRG neurons were first suggested by selective depolarization of C fibers by kainate (1, 24), and by the microscopic demonstration of NR1 receptors in terminals of primary afferents in the superficial laminae of the dorsal horn (45). In subsequent work on a variety of preparations, presynaptic kainate, AMPA and NMDA receptors have been proposed to modulate the release of glutamate from terminals of central processes of DRG neurons (10, 40, 42, 43, 70).

The expression of PIGR in terminals of central processes of DRG neurons was surveyed by confocal and electron microscopy in the dorsal horn of the adult rats. The results showed which subunits of kainate, AMPA, and NMDA receptors are expressed in terminals of myelinated and/or unmyelinated afferents. The functional types of terminals that express the different subunits were inferred on the basis of their morphology and simultaneous labeling with functional markers.

The KA1 and KA2 subunits of the kainate receptor are expressed in a variety of terminals, including nociceptive afferents to laminae I and II (Lucifora *et al.*, in preparation). Of the three subunits of the low-affinity kainate receptor, GluR5 is also expressed in terminals of A- and C fibers (35, 59, 80, 82), and it is the critical subunit for activation of the heteromeric complex of presynaptic kainate receptors (41). The degree to which high- and low-affinity kainate receptor subunits may be coexpressed in the same presynaptic terminals has not been tested, but GluR5 and KA2 form the predominant heteromeric pair in postsynaptic kainate receptors in the hippocampus (34). Activation of presynaptic kainate receptors on central endings of DRG neurons may result in an increase (42) or a decrease (40) of glutamate release,

probably depending on the concentration of the agonist, Ca^{2+} influx at Ca^{2+}-permeable receptors, and other factors not yet adequately explored (21, 27, 34).

Although all AMPA receptor subunits may be synthesized by DRG neurons (20, 43, 47, 71, 75), evidence for central transport has been provided mainly for the GluR3 and GluR4 subunits (47), suggesting a selective expression of Ca^{2+}-permeable homomeric or heteromeric complexes of presynaptic AMPA receptors in central afferents. While presynaptic GluR4 is primarily expressed in terminals in superficial laminae (I and II) of the dorsal horn, presynaptic GluR3 is expressed primarily in deeper laminae which receive low-threshold mechanosensory input (47). In the only study thus far focused on the effects of activation of AMPA receptors in central terminals of DRG neurons in rats, excitatory postsynaptic currents in dorsal horn neurons of spinal cord slices were suppressed, in the presence of a cocktail of pharmacological agents ensuring that the decrease in glutamate release was mediated presynaptically (43).

Central transport of NR1 from DRG neurons was first reported in C fibers by Liu et al (45). Since glutamate is released by terminals of central processes of DRG neurons, presynaptic NMDA receptors, like kainate and AMPA receptors in the same terminals, were interpreted as autoreceptors. More recent work has demonstrated expression of presynaptic NR1 in a large proportion of terminals of A fibers as well (48). Different subtypes of the NR2 subunit may colocalize with NR1 in different types of DRG neurons and their central terminals (13, 49, 52) to form heteromeric complexes which require NR1 and one subtype of NR2 to be functional (54). Presynaptic NMDA receptors may facilitate release of substance P from C fibers (46, 50, 51). In contrast, activation of the same receptors inhibits glutamate release from central terminals of DRG neurons (10).

As the presence of PIGR on central terminals of DRG neurons has become validated by the evidence summarized above, several questions arise. Among these, three are most prominent: 1) What is the source of glutamate that activates PIGR? 2) What is the mechanism by which activation of PIGR may modulate release of glutamate? 3) What is the role of PIGR in the context of modulation of sensory input to the spinal cord?

1) Glutamate which activates PIGR can spill over from the synaptic cleft at glutamatergic synapses. Theoretical and experimental studies have determined the diffusion rate of glutamate in synaptic complexes in hippocampus and cerebellum (9, 27, 29). This rate is compatible with activation of PIGR by spilled over glutamate from the same, or immediately adjacent, terminal. However, this possibility has not been tested experimentally for central terminals of DRG neurons. Many factors besides diffusion rate, may influence the efficacy of spilled over glutamate in activating PIGR, including the distance of the receptors from the synaptic cleft, the affinity of the receptors, the density of glutamate transporter molecules, the rate of desensitization, and other presynaptic mechanisms that may variably determine synaptic strength in different synapses (8).

Another potential source of glutamate for PIGR activation is astroglia. The classical view of astrocytes as passive supporting elements for neurons has been repla-

ced by the recognition that neural transmission may be markedly affected by astrocytes (56). Astrocytes express receptors for several mediators and can respond in a graded manner to neuronal activity (55, 65). Furthermore, an increase in Ca^{2+} propagates in regenerative non-decremental waves that may involve adjacent astrocytes (7). This Ca^{2+} elevation, in turn, elicits glutamatergic currents in adjacent neurons (58). The experimental evidence suggests that glutamate released by astrocytes, possibly by a vesicular mechanism (12), affects neuronal transmission by modulating the probability of synaptic release of glutamate, hence it may involve presynaptic glutamate receptors (5, 6, 17, 31, 60).

2) The mechanisms underlying modulation of glutamate release by activation of PIGR in central terminals of DRG neurons remain to be elucidated. Two, non-mutually exclusive, hypotheses are commonly proposed (27, 40). One is that release of glutamate is modulated by depolarization of terminals, in a manner not unlike that proposed for GABA-mediated presynaptic inhibition (see above). This hypothesis originated from studies on hippocampal preparations and cerebellar mossy fibers showing that depression of synaptic transmission induced by activation of presynaptic kainate receptors is correlated with: a) increased excitability of fiber terminals, and b) reduced Ca^{2+} influx accompanying an action potential in mossy fiber terminals (38, 39, 73). A similar conclusion was also drawn from spinal cord slices of rat pups in which application of AMPA or NMDA depolarizes central terminals of DRG neurons by way of presynaptic receptors on these terminals (10, 43).

The other hypothesis is that PIGR modulate release of glutamate by an intracellular G protein-mediated, signaling path that might affect other channels or intermediate intracellular signaling molecules (19). A metabotropic role for kainate receptors was proposed on the basis of the activation by these receptors of pertussin toxin-sensitive G-proteins and kinases, and of slow after-hyperpolarization generated by burst of action potentials (53, 67). In DRG neurons, activation of the GluR5 subunit of the kainate receptor is required for a metabotropic cascade, independent of the opening of the ion channel (68). Activation of the NMDA receptor opens the route to the Ras/Rap1/MAPK pathway leading to ERK-1 and ERK-2 kinases, possibly via the involvement of a Ras-GTPase activating protein, SynGAP, which is highly expressed at excitatory synapses (36, 64). Schenk and Matteoli (72) have advanced some speculations on how a metabotropic cascade, initiated by AMPA-activated channels, might modulate the release of neurotransmitter in a synaptic terminal. Since AMPA receptor subunits can activate MAPK, as is the case for kainate and NMDA receptors, and also inhibit protein kinase A (61), via the intermediary of a G_i protein (78), they propose a double effect of presynaptic AMPA receptor activation onto glutamate release via two different metabotropic pathways. Both pathways involve phosphorylation of synapsin I, a presynaptic substrate for both kinases, which regulates fusion of synaptic vesicles at the presynaptic membrane and exocytosis. Activation of MAPK, however, would result in increased transmitter release, while inhibition of protein kinase A would have the opposite effect.

3) A role for PIGR in the context of modulation of sensory input to the spinal cord is, at present, only a matter of speculation. If it will be confirmed that the predominant effect of PIGR activation is to decrease glutamate release, these receptors may provide a more generalized, and perhaps more powerful, mechanism acting in parallel with GABA-mediated presynaptic inhibition. In fact, PIGR may be the main presynaptic inhibitory mechanism in Aδ- and C fibers in spinal cord, which may not be affected by GABA-mediated presynaptic inhibition (see above). Were this the case, PIGR may have a prominent role in the control of nociception.

Expression of PIGR, however, is not limited to central terminals of DRG neurons, nor is it limited to glutamate-releasing spinal neurons. Recent work has shown that about a third of GABA-ergic neurons in superficial laminae of the dorsal horn of the spinal cord express PIGR (Lu *et al.*, in press). If activation of PIGR in these inhibitory neurons results in decrease of GABA release, the net result of PIGR activation in a network of neurons releasing different transmitters in the laminae of termination of Aδ- and C fibers can vary and even contribute to central sensitization and hyperalgesia.

SUMMARY

Sensory input from peripheral nerves to the dorsal horn of the spinal cord is mediated by a variety of agents released by the central terminals of dorsal root ganglion (DRG) neurons. These include, but are not limited to, amino acids, especially glutamate, peptides and purines. The unraveling of the mechanisms of synaptic transmission by central terminals of DRG neurons has to take into account various ways in which the message from the periphery can be modulated at the level of the first central synapse. These include postsynaptic and presynaptic mechanisms. Homomeric and heteromeric complexes of receptor subunits for the different transmitters released by DRG neurons and interneurons, clustered at the postsynaptic site of central synapses, can be expressed in different combinations and their rate of insertion into the postsynaptic membrane is activity-regulated. Inhibitory mechanisms are an important part of central modulation, especially via presynaptic inhibition, currently believed to involve GABA released by inhibitory intrinsic neurons.

Recent work has established the occurrence of another way by which sensory input can be modulated, i.e. the expression of presynaptic ionotropic and metabotropic receptors in central terminals of DRG neurons. Microscopic evidence for the expression, in these terminals, of various subunits of ionotropic glutamate receptors documents the selective expression of glutamate receptors in functionally different DRG afferents. Electrophysiological and pharmacological data suggest that activation of presynaptic ionotropic glutamate receptors in central terminals of DRG neurons may result in inhibition of release of glutamate by the same terminals. Glutamate activating presynaptic receptors may spill over from the same or adjacent synapses, or may be released by processes of astroglial cells surrounding synaptic terminals.

The wide expression of presynaptic ionotropic glutamate receptors, especially in superficial laminae of the dorsal horn, where Aδ- and C fibers terminate, provides an additional or alternative mechanism, besides GABA-mediated presynaptic inhibition, for the modulation of glutamate release by these fibers. Since, however, presynaptic ionotropic glutamate receptors are also expressed in terminals of GABAergic intrinsic interneurons, a decrease of GABA release resulting from activation of these receptors in the same laminae, may also play a role in central sensitization and hyperalgesia.

REFERENCES

1. AGRAWAL, S.G. AND EVANS, R.H. The primary afferent depolarizing action of kainate in the rat. *Br. J. Pharmacol.*, **87**: 345-355., 1986.
2. ALVAREZ, F.J. Anatomical basis for presynaptic inhibition of primary sensory fibers. In: RUDOMIN, P., ROMO R. AND MENDELL, L.M. (Eds.) *Presynaptic Inhibition and Neural Control.* New York: Oxford University Press 1998.
3. ALVAREZ, F.J., TAYLOR-BLAKE, B., FYFFE, D.E., DE BLAS, A.L. AND LIGHT, A.R. Distribution of immunoreactivity for the beta, 2 and beta, 3 subunits of the $GABA_A$ receptor in the mammalian spinal cord. *J. Comp. Neurol.*, **365**: 392-412, 1996.
4. ALVAREZ-LEEFMANS, F.J., NANI, A. AND MÁRQUEZ, S. Chloride transport, osmotic balance, and presynaptic inhibition. In: RUDOMIN, P. ROMO R. AND MENDELL L.M. (Eds.) *Presynaptic Inhibition and Neural Control.* New York: Oxford University Press 1998.
5. ARAQUE, A., CARMIGNOTO, G. AND HAYDON P.G. Dynamic signaling between astrocytes and neurons. *Am. Rev. Physiol.*, **63**: 795-813, 2001.
6. ARAQUE, A., SANZGIRI, R.R., PARPURA, V. AND HAYDON P.G. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J. Neurosci.*, **18**: 822-6829, 1998.
7. ARAQUE, A., SANZGIRI, R.R., PARPURA, V. AND HAYDON, P.G. Astrocyte-induced modulation of synaptic transmission. *Can. J. Physiol. Pharmacol.*, **77**: 699-706, 1999.
8. ATWOOD, H.L. AND KARUNANITHI, S. Diversification of synaptic strength: presynaptic elements. *Nature Rev. Neurosci.*, **3**: 497-516, 2002.
9. BARBOUR B. An evaluation of synapse independence. *J. Neurosci.*, **21**: 7969-7984, 2001.
10. BARDONI, R., TORSNEY, C., TONG, C.-T., PRANDINI, M. AND MACDERMOTT A.B. Presynaptic NMDA receptors modulate glutamate release from primary sensory neurons in rat spinal cord dorsal horn. *J. Neurosci.*, **24**: 2774-2778, 2004.
11. BERNARDI, P.S., VALTSCHANOFF, J.G., WEINBERG, R.J., SCHMIDT, H.H.H. AND RUSTIONI, A. Synaptic interactions between primary afferent terminals and GABA and nitric oxide-synthesizing neurons in superficial laminae of the rat spinal cord. *J. Neurosci.*, **15**: 1361-1371, 1995.
12. BEZZI, P., GUNDERSEN, V., GALBETE, J.L., SEIFERT, G., STEINHÄUSER, C., PILATI, E. AND VOLTERRA, A. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. *Nature Neurosci.*, **7**: 613-620, 2004.
13. BOYCE, S., WYATT, A., WEBB, J.K., O'DONNELL, R., MASON, G., RIGBY, M., SIRINATHSINGHJI, D., HILL R.G. AND RUPNIAK, N.M.J. Selective NMDA NR2B antagonists induce antinociception without motor disfunction. *Neuropharmacology*, **38**: 611-623, 1999.
14. CALVILLO, O., MADRID, J. AND RUDOMIN, P. Presynaptic depolarization of unmyelinated primary afferent fibers in the spinal cord of the cat. *Neuroscience*, **7**: 1386-1400, 1982.

15. CARLTON, S.M., HARGETT, G.L. AND COGGESHALL, R.E. Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. *Neurosci. Lett.*, **197**: 25-28, 1995.
16. CARLTON, S.M., HARGETT, G.L. AND COGGESHALL, R.E. Plasticity in alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor subunits in the rat dorsal horn following deafferentation. *Neurosci. Lett.*, **242**: 21-24, 1998.
17. CARMIGNOTO, G. Bidirectional communication between neurons and astrocytes. *Progr. Neurobiol.*, **62**: 561-581, 2000.
18. CARSTENS, E., TULLOCH, I., ZIEGLGÄNSBERGER, W. AND ZIMMERMANN, M. Presynaptic excitability changes induced by morphine in single cutaneous afferent C- and A-fibers. *Pflügers Arch.*, **379**: 143-147, 1979.
19. CASADO, M., DIEUDONNE, S. AND ASCHER, P. Presynaptic *N*-methyl-D-aspartate receptors at the parallel fiber-Purkinje cell synapse. *Proc. Natl. Acad. Sci. USA*, **97**: 11593-11597, 2000.
20. CHAMBILLE, I. AND RAMPIN, O. AMPA glutamatergic receptor-immunoreactive subunits are expressed in lumbosacral neurons of the spinal cord and neurons of the dorsal root and pelvic ganglia controlling pelvic functions in the rat. *Brain Res.*, **933**: 66-80, 2002.
21. COCHILLA, A. AND ALFORD, S. NMDA receptor-mediated control of presynaptic calcium and neurotransmitter release. *J. Neurosci.*, **19**: 193-205, 1999.
22. COGGESHALL, R.E. AND CARLTON, S.M. Ultrastructural analysis of NMDA, AMPA and kainate receptors on unmyelinated and myelinated axons in the periphery. *J. Comp. Neurol.*, **391**: 78-86, 1998.
23. DAVIDSON, E.M. AND CARLTON, S.M. Intraplantar injection of dextrorphan, ketamine or memantine attenuates formalin-induced behaviors. *Brain Res.*, **785**: 136-142, 1998.
24. DAVIES, J., EVANS, R.H., FRANCIS, A.A. AND WATKINS, J.C. Excitatory amino acid receptors and synaptic excitation in the mammalian central nervous system. *J. Physiol. Paris*, **75**: 641-654, 1979.
25. DU, J., KOLTZENBURG, M. AND CARLTON, S.M. Glutamate-induced excitation and sensitization of nociceptors in rat glabrous skin. *Pain*, **89**: 187-198, 2001.
26. DUDEL, J. AND KUFFLER, S.W. Presynaptic inhibition at the crayfish neuromuscular junction. *J. Physiol. Lond.*, **155**: 543-562, 1961.
27. ENGELMAN, H.S. AND MACDERMOTT, A.B. Presynaptic ionotropic receptors and control of transmitter release. *Nature Rev. Neurosci.*, **5**: 135-145, 2004.
28. FITZGERALD, M. AND WOOLF, C.J. Effects of cutaneous nerve and intraspinal conditioning on C-fiber afferent terminal excitability in decerebrate spinal rats. *J. Physiol. Lond.* **318**: 25-39, 1981.
29. FRANKS, K.M., BARTOL, T.M. JR. AND SEJNOWSKI, T.J. A Monte Carlo model reveals independent signaling at central glutamatergic synapses. *Biophys. J.*, **83**: 2333-2348, 2002.
30. GRAHAM, B. AND REDMAN, S. A simulation of action potentials in synaptic boutons during presynaptic inhibition. *J. Neurophysiol.*, **71**: 538-549, 1994.
31. HAYDON, P.G. Glia: listening and talking to the synapse. *Nature Rev. Neurosci.*, **2**: 185-193, 2001.
32. HENTALL, I.D. AND FIELDS, H.L. Segmental and descending influences on intraspinal thresholds of single C fibers. *J. Neurophysiol.*, **42**: 1527-1537, 1979.
33. HUETTNER, J.E. Glutamate receptor channel in rat DRG neurons: activation by kainate and quisqualate and blockade of desensitization by Con A. *Neuron*, **5**: 255-266, 1990.
34. HUETTNER, J.E. Kainate receptors and synaptic transmission. *Progr. Neurobiol.*, **70**: 387-407, 2003.
35. HWANG, S.J., PAGLIARDINI, S., RUSTIONI, A. AND VALTSCHANOFF, J.G. Presynaptic kainate receptors in primary afferents to the superficial laminae of the rat spinal cord. *J. Comp. Neurol.*, **436**: 275-289, 2001.

36. IMAMURA, Y., MATSUMOTO, N., KONDO, S., KITAYAMA, H. AND NODA, M. Possible involvement of Rap1 and Ras in glutamatergic synaptic transmission. *NeuroReport*, **14**: 1203-1207, 2003.
37. JIA, H., RUSTIONI, A. AND VALTSCHANOFF, J.G. Metabotropic glutamate receptors in superficial laminae of the rat dorsal horn. *J. Comp. Neurol.*, **410**: 627-642, 1999.
38. KAMIYA, H. AND OZAWA, S. Kainate receptor-mediated inhibition of presynaptic Ca^{2+} influx and EPSPS in area CA1 of the rat hippocampus. *J. Physiol. Lond.*, **509**: 833-845, 1998.
39. KAMIYA, H. AND OZAWA, S. Kainate receptor-mediated presynaptic inhibition at the mouse hipocampal mossy fibre synapse. *J. Physiol. Lond.*, **523**: 653-665, 2000.
40. KERCHNER, G.A., WILDING, T.J., LI, P., ZHUO, M. AND HUETTNER, J.E. Presynaptic kainate receptors regulate spinal sensory transmission. *J. Neurosci.*, **21**: 59-66, 2001.
41. KERCHNER, G.A., WILDING, T.J., HUETTNER, J.E. AND ZHUO, M. Kainate receptor subunits underlying presynaptic regulation of transmitter release in the dorsal horn. *J. Neurosci.*, **22**: 8010-8017, 2002.
42. LEE, C.J., ENGELMAN, H.S. AND MACDERMOTT, A.B. Activation of kainate receptors on rat sensory neurons evokes action potential firing and may modulate transmitter release. *Ann. N.Y. Acad. Sci.*, **868**: 546-549, 1999.
43. LEE, C.J., BARDONI, R., TONG, C.K., ENGELMAN, H.S., JOSEPH, D.J., MAGHERINI, P.C. AND MACDERMOTT, A.B. Functional expression of AMPA receptors on central terminals of rat dorsal root ganglion neurons and presynaptic inhibition of glutamate release. *Neuron*, **35**: 135-146, 2002.
44. LI, H., OHISHI, H., KINOSHITA, A., SHIGEMOTO, R., NOMURA, S. AND MIZUNO, N. Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat. *Neurosci. Lett.*, **223**: 153-156, 1997.
45. LIU, H., WANG, H., SHENG, M., JAN, Y.N. AND BASBAUM, A.I. Evidence for presynaptic *N*-methyl-D-aspartate autoreceptors in the spinal cord dorsal horn. *Proc. Natl. Acad. Sci. USA*, **91**: 8383-8387, 1994.
46. LIU, H., MANTYH, P.W. AND BASBAUM, A.I. NMDA-receptor regulation of substance P release from primary afferent nociceptors. *Nature*, **386**: 721-724, 1997.
47. LU, C.-R., HWANG, S.J., PHEND, K.D., RUSTIONI, A. AND VALTSCHANOFF, J.G. Primary afferent terminals in spinal cord express presynaptic AMPA receptors. *J. Neurosci.*, **22**: 9522-9529, 2002.
48. LU, C.-R., HWANG, S.J., PHEND, K.D., RUSTIONI, A. AND VALTSCHANOFF, J.G. Primary afferent terminals that express presynaptic NR1 in rats are mainly from myelinated, mechanosensitive fibers. *J. Comp. Neurol.*, **460**: 191-202, 2003.
49. MA, Q.P. AND HARGREAVES, R.J. Localization of *N*-methyl-D-aspartate NR2B subunits on primary sensory neurons that give rise to small-caliber sciatic nerve fibers in rats. *Neuroscience*, **101**: 699-707, 2000.
50. MALCANGIO, M., FERNANDES, K. AND TOMLISON, D.R. NMDA receptor activation modulates evoked release of substance P from rat spinal cord. *Br. J. Pharmacol.*, **125**: 1625-1626, 1998.
51. MARVIZÓN, J.C.G., MARTINEZ, Z., GRADY, E.F., BURNETT, N.W. AND MAYER, E.A. Neurokinin, 1 receptor internalization in spinal cord slices induced by dorsal root stimulation is mediated by NMDA receptors. *J. Neurosci.*, **17**: 8129-8136, 1997.
52. MARVIZÓN, J.C.G., MCROBERTS, J.A., ENNES, H.S., SONG, B., WANG, X., JINTON, L., CORNELIUSSEN, B. AND MAYER, E.A. Two N-methyl-D-aspartate receptors in rat dorsal root ganglia with different subunit composition and localization. *J. Comp. Neurol.*, **446**: 325-341, 2002.

53. MELYAN, Z., WHEAL, H.V. AND LANCASTER, B. Metabotropic-mediated kainate receptor regulation of I(sAHP) and excitability in pyramidal cells. *Neuron*, **34**: 107-114, 2002.
54. MONYER, H., SPRENGEL, R., SCHOEPFER, R., HERB, A., HIGUCHI, M., LOMELI, H., BURNASHEV, N. AND SEEBURG, P.H. Heteromeric NMDA receptors: molecular and functional distinction of subtypes. *Science*, **256**: 1217-1221, 1992.
55. MURPHY, T.H., BLATTER, L.A., WIER, W.G. AND BARABAN, J.M. Rapid communication between neurons and astrocytes in primary cortical cultures. *J. Neurosci.*, **13**: 2672-2679, 1993.
56. NEWMAN, E.A. New roles for astrocytes: regulation of synaptic transmission. *Trends Neurosci.*, **26**: 536-542, 2003.
57. NIELSEN, T.A., DIGREGORIO, D.A. AND ANGUS SILVER, R. Modulation of glutamate mobility reveals the mechanism underlying slow-rising AMPAR EPSCs and the diffusion coefficient in the synaptic cleft. *Neuron*, **42**: 757-771, 2004.
58. PARPURA, V. AND HAYDON, P.G. From the cover: physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc. Natl. Acad. Sci. USA*, **97**: 8629-8634, 2000.
59. PARTIN, K.M., PATNEAU, D.K., WINTERS, C.A., MAYER, M.L. AND BUONANNO, A. Selective modulation of desensitization at AMPA versus kainate receptors by cyclothiazide and concanavalin A. *Neuron*, **11**: 1069: 1082, 1993.
60. PASTI, L., ZONTA, M., POZZA, T., VICINI, S. AND CARMIGNOTO, G. Cytosolic calcium oscillations in astrocytes may regulate exocytotic release of glutamate. *J. Neurosci.*, **21**: 477-484, 2001.
61. PERKINTON, M., SIHRA, T. AND WILLIAMS, R. Ca^{2+}-permeable AMPA receptors induce phosphorylation of cAMP response element-binding protein through a phosphatidylinositol, 3-kinase-dependent stimulation of the mitogen-activated protein kinase signaling cascade in neurons. *J. Neurosci.*, **19**: 5861-5874, 1999.
62. PETRALIA, R.S., WANG, Y.-Z. AND WENTHOLD, R.J. Histological and ultrastructural localization of the kainate receptor subunits, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. *J. Comp. Neurol.*, **349**: 85-110, 1994a.
63. PETRALIA, R.S., WANG, Y.-Z. AND WENTHOLD, R.J. Light and electron microscope distribution of the NMDA receptor subunit NMDAR1 in the rat nervous system using a selective anti-peptide antibody. *J. Neurosci.*, **14**: 667-696, 1994b.
64. PLÁTENIK, J., KURAMOTO, N. AND YONEDA, Y. Molecular mechanisms associated with long-term consolidation of the NMDA signals. *Life Sci.*, **67**: 335-364, 2000.
65. PORTER, J.T. AND MCCARTHY, K.D. Hippocampal astrocytes *in situ* respond to glutamate released from synaptic terminals. *J. Neurosci.*, **16**: 5073-5081, 1996.
66. RANDIĆ, M. Presynaptic effects of γ-aminobutyric acid and substance P on intraspinal single cutaneous C- and A-fibers. In: BROWN, A.G. AND RÉTHELYI M. (Eds.) *Spinal Cord Sensation*. Scottish Academic Press, Edinburgh, 1981.
67. RODRIGUEZ-MORENO, A., LOPEZ-GARCIA, J.C. AND LERMA, J. Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. *Proc. Natl. Acad. Sci. USA*, **97**: 1293-1298, 2000.
68. ROZAS, J.L., PATERNAIN, A.V. AND LERMA, J. Noncanonical signaling by ionotropic kainate receptors. *Neuron*, **39**: 543-553, 2003.
69. RUDOMIN, P. AND SCHMIDT, R. Presynaptic inhibition in the vertebrate cord revisited. *Exp. Brain Res.*, **129**: 1-37, 1999.
70. RUSSO, R.E., DELGADO-LEZAMA, R. AND HOUNSGAARD, J. Dorsal root potential produced by a TTX-insensitive micro-circuitry in the turtle spinal cord. *J. Physiol. Lond.*, **528**: 115-122, 2000.

71. SATO, K., KIYAMA, H., PARK, H.T. AND TOHYAMA, M. AMPA, KA and NMDA receptors are expressed in the rat DRG neurons. *NeuroReport*, **4**: 1263-1265, 1993.
72. SCHENK, U. AND MATTEOLI, M. Presynaptic AMPA receptors: more than just ion channels? *Biol. Cell*, **96**: 257-260, 2004.
73. SCHMITZ, D., FRERKING, M. AND NICOLL, R.A. Synaptic activation of presynaptic kainate receptors on hippocampal mossy fiber synapses. *Neuron*, **27**: 327-338, 2000.
74. SHIGEMOTO, R., ONISHI, H., NAKANISHI, S. AND MIZUNO, N. Expression of the mRNA for the rat NMDA receptor (NMDAR1) in the sensory and autonomic ganglion neurons. *Neurosci. Lett.*, **144**: 229-232, 1992.
75. TACHIBANA, M., WENTHOLD, R.J., MORIOKA, H. AND PETRALIA, R.S. Light and electron microscopic immunocytochemical localization of AMPA-selective glutamate receptors in the rat spinal cord. *J. Comp. Neurol.*, **344**: 431-454, 1994.
76. TOWERS, S., PRINCIVALLE, A., BILLINTON, A., EDMUND, M., BETTLER, B., URBAN, L., CASTRO-LOPEZ, J. AND BOWERY, N.G. $GABA_B$ receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. *Eur. J. Neurosci.*, **12**: 3201-3210, 2000.
77. USHIDA, T., RANI, T., KAWASAKI, M., IWATSU, O. AND YAMAMOTO, H. Peripheral administration of an *N*-methyl-D-aspartate receptor antagonist (MK-801) changes dorsal horn neuronal response in rats. *Neurosci. Lett.*, **260**: 89-92, 1999.
78. WANG, Y., SMALL, D.L., STANIMIROVIC, D.B., MORLEY, P. AND DURKIN, J.P. AMPA receptor-mediated regulation of a G_i-protein in cortical neurons. *Nature*, **389**: 502-504, 1997.
79. WATANABE, M., MISHINA, M. AND INOUE, Y. Distinct gene expression of the N-methyl-D-aspartate receptor channel subunit in peripheral neurons of the mouse sensory ganglia and adrenal gland. *Neurosci. Lett.*, **165**: 183-186, 1994.
80. WILDING, T.J. AND HUETTNER, J.E. Functional diversity and developmental changes in rat neuronal kainate receptors. *J. Physiol. Lond.*, **532**: 411-421, 2001.
81. WILLIS, W.D. JR. Dorsal root potentials and dorsal root reflexes: a double-edged sword. *Exp. Brain Res.*, **124**: 395-421, 1999.
82. YOUN, D.H. AND RANDIĆ, M. Modulation of excitatory synaptic transmission in the spinal substantia gelatinosa of mice deficient in the kainate receptor GluR5 and/or GluR6 subunit. *J. Physiol. Lond.*, **555**: 683-698, 2004.
83. ZHOU, S., BONASERA, L. AND CARLTON, S.M. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. *NeuroReport*, **7**: 895-900, 1996.