Neuronal Plasticity: Increasing the Gain in Pain

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We describe those sensations that are unpleasant, intense, or distressing as painful. Pain is not homogeneous, however, and comprises three categories: physiological, inflammatory, and neuropathic pain. Multiple mechanisms contribute, each of which is subject to or an expression of neural plasticity—the capacity of neurons to change their function, chemical profile, or structure. Here, we develop a conceptual framework for the contribution of plasticity in primary sensory and dorsal horn neurons to the pathogenesis of pain, identifying distinct forms of plasticity, which we term activation, modulation, and modification, that by increasing gain, elicit pain hypersensitivity.

Physiological pain is an essential early warning device that alerts us to the presence in the environment of damaging stimuli. This is the pain we experience in response to a needle prick. All living organisms need to be able to react to noxious stimuli, and a major evolutionary drive for the development of a plastic nervous system might have been the acquisition of the capacity to detect and remember danger.

Physiological pain is initiated by specialized sensory nociceptor fibers innervating peripheral tissues and activated only by noxious stimuli. The sensory inflow generated by nociceptors activates neurons in the spinal cord which project to the cortex via a relay in the thalamus, eliciting pain. The nociceptor input also activates reflex withdrawal, an increase in arousal as well as emotional, autonomic, and neurohumeral responses.

Activation of the Pain System

Physiological pain (Fig. 1) starts in the peripheral terminals of nociceptors with the activation of nociceptive transducer receptor/ion channel complexes, which generate depolarizing currents in response to noxious stimuli (Fig. 2). Transducer proteins that respond to extrinsic or intrinsic irritant chemical stimuli (VR1, DRASIC, P2X3) are selectively expressed in sensory neurons (*1*). Noxious heat transducers include the vanilloid receptors VR1 and VRL1 (*2*). A transducer for noxious mechanical stimuli has not been identified, although studies in *Caenorhabditis elegans* suggest that it may belong to the mDeg ion channel family (*3*).

Transduction is followed, if the current is sufficient, by initiation of action potentials. The action potentials are conducted to the central nervous system (CNS), where their invasion of central nociceptor terminals in the spinal cord initiates transmitter release. Fast excitatory synaptic transmission in pain pathways is mediated by glutamate acting on AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) and kainate ligand-gated ion channels (Fig. 3A) (*4*). The excitation is focussed by segmental and descending activation of inhibitory neurons, most of which co-release glycine and γ-aminobutyric acid (GABA) (*5*).

Activation-Dependent Plasticity

Activation of nociceptive pathways is subject to activity-dependent plasticity, which manifests as a progressive increase in the response of the system to repeated stimuli.

Activation-dependent plasticity in nociceptor terminals: Autosensitization. The high threshold of nociceptor terminals can be decreased either by changes in the transducers themselves as a result of prior activation, a phenomenon we term autosensitization, or by an increase in the excitability of the terminal membrane, which we call heterosensitization, that is initiated by sensitizing stimuli that do not activate the transducers.

Autosensitization of vanilloid receptors upon their repeated activation by heat, capsaicin, or protons is observed electrophysiologically (*6*) and parallels changes in heat responsive nociceptors and pain in humans (*7*). The changes are rapid in onset, substantial, and readily reversible, and may represent conformational changes in the protein induced by heat, or alterations secondary to calcium entry through the transducer (*8*).

Activation-dependent plasticity in dorsal horn neurons: Windup. Low-frequency activation of nociceptors by mild noxious stimuli generates fast excitatory postsynaptic potentials (EPSPs) that signal the onset, duration, intensity, and location of the stimulus (Fig. 3A). Higher frequency inputs generated by intense or sustained noxious stimuli result in the co-release of neuromodulators (*9*) as well as glutamate, producing slow EPSPs lasting tens of seconds (Fig. 3B) (*10*). This provides for temporal summation (*11*), and the resulting cumulative depolarization is boosted by additional *N*-methyl-D-aspartate (NMDA) receptor current on removal of the Mg^{2+} blockade of the channels (*12*). Depolarization also activates voltage-gated calcium currents triggering plateau potentials mediated by calcium-activated nonselective cation channels (Fig. 3B) (*13*). The net effect is a windup of action potential discharge.

Clinical Pain

Inflammatory pain is initiated by tissue damage/inflammation and neuropathic pain by nervous system lesions. Both are characterized by hypersensitivity at the site of damage and in adjacent normal tissue. Pain may appear to arise spontaneously, stimuli that would never normally produce pain begin to do so (allodynia) and noxious stimuli evoke a greater and more prolonged pain (hyperalgesia) (*14*).

Inflammatory pain hypersensitivity usually returns to normal if the disease process is controlled. Neuropathic pain persists long after the initiating event has healed and is an expression of pathological operation of the nervous system rather than a reaction to pathology.

The plasticity responsible for clinical pain hypersensitivity has two general forms, modulation and modification (Fig. 1). Modulation represents reversible changes in the excitability of primary sensory and central neurons mediated by posttranslational alterations induced in receptors/ion channels by activation of intracellular signal transduction cascades. Modification represents long-lasting alterations in the expression of transmitters/receptors/ion channels or in the structure, connectivity and survival of neurons, such that the system is grossly modified, distorting its normal stimulus-response characteristics.

Modulation of the Pain System

The major mechanism responsible for modulation is phosphorylation of receptor/ion channels, or associated regulatory proteins, altering intrinsic functional properties or cell-surface expression of channels in primary sensory and dorsal horn neurons. The intracellular pathways involve interactions of serine/threonine and tyrosine kinase signaling cascades.

Modulation of the peripheral terminals of nociceptors: Heterosensitization. An increase

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in the excitability of the nociceptor terminal membrane will reduce the amount of depolarization required to initiate an action potential discharge. This modulation occurs on exposure of the terminal to sensitizing agents and contributes to peripheral sensitization. The sensitizing agents include inflammatory mediators (PGE2, 5-HT, bradykinin, epinephrine, adenosine) and neurotrophic factors (NGFs) released during tissue damage or by inflammatory cells that may or may not activate the terminal, but which also sensitize it to subsequent stimuli (*15, 16*). The modulation is the result of the parallel activation of intracellular kinases by G protein coupled– and tyrosine kinase membrane-bound receptors (*17*) activating protein kinase A (*18*) or protein kinase C ε (*19, 20*), phosphorylating a tetrodotoxin (TTX) resistant sensory neuron–specific sodium ion channel SNS and possibly VR1 (*21*). Phosphorylation of SNS alters its properties, including activation threshold and rate of activation/inactivation, and increases the magnitude of the sodium current to depolarization (*22, 23*). SNS knockdown and knockout (*24, 25*) indicate that it contributes to inflammatory pain hypersensitivity. Because different sensitizing signal molecules acting on different receptors effect the same end result, inhibiting a single sensitizing agent is unlikely to completely eliminate peripheral sensitization.

Modulation of nociceptive synaptic transmission: Central sensitization. Modulation in central pain pathways is triggered by peripheral nociceptor input and results in enhanced responsiveness of pain transmission neurons, which either outlasts the initiating input or requires a low-level peripheral drive to maintain it (*26, 27*). Modulation involves activation of intracellular signaling cascades leading to facilitated excitatory synaptic responses and depressed inhibition, thereby amplifying responses to noxious and innocuous inputs. The changes may be restricted to the activated synapse (homosynaptic) or spread to adjacent synapses (heterosynaptic). Most excitatory input to pain pathway neurons is subthreshold, and increased gain results in recruitment of these inputs to the output of the neurons, causing them to fire to normally ineffective inputs (*28, 29*). These changes constitute central sensitization and are responsible for pain produced by low-threshold afferent inputs and the spread of hypersensitivity to regions beyond injured tissue (*30, 31*).

Activity-dependent enhancement of transmission is common at excitatory synapses throughout the CNS and is broadly separable into NMDA receptor–dependent and NMDA receptor–independent types. Homosynaptic potentiation of AMPA-receptor responses at synapses on dorsal horn neurons is produced experimentally by brief duration, high-frequency (100 Hz) nociceptor stimulation and is dependent upon NMDA receptor activation (*32*). The requirement for high-frequency afferent input and NMDA receptors is like that of long-term potentiation (LTP) in CA1 hippocampal neurons (*33*). At CA1 synapses, LTP is initiated by a signaling cascade involving enhancement of NMDA receptor function by the tyrosine kinase Src, raised intracellular [Ca^{2+}], activation of calcium/calmodulin-dependent kinase II (CaMKII) and PKC, and enhanced AMPA channel conductance and/or cell-surface expression (*34, 35*). In dorsal horn neurons, NMDA receptors are known to be up-regulated by Src (*36*) and regulated AMPA channel insertion dependent upon PKC has been demonstrated (*37*). Thus, homosynaptic potentiation in the dorsal horn is likely to proceed via mechanisms analogous to LTP in CA1. However, given that expression of CaMKII is low in the dorsal horn, another calcium-dependent serine/threonine kinase, such as PKC, may play an equivalent role.

Because nociceptors do not usually fire at high frequencies, homosynaptic potentiation may be limited to very intense stimuli. Heterosynaptic potentiation, because it can be initiated by low-frequency nociceptor inputs (1.0 Hz), is a more prominent feature of synaptic modulation in the dorsal horn, enhancing synapses not activated by the conditioning input, evoking dispersed hypersensitivity as revealed by increases in receptive field size and recruitment of novel inputs (*38, 39*). Whenever C-fiber nociceptors are activated more than transiently, they induce central sensitization and this NMDA receptor–mediated phenomenon is a major component of inflammatory and neuropathic pain (*40, 41*). Involvement of NMDA receptors is attributable to two mechanisms (Fig. 3C). First, a cumulative depolarization produced by summation of nociceptor-evoked slow synaptic potentials (*42*) leading to suppression of Mg^{2+} blockade of NMDA channels. Second, enhanced NMDA channel gating through convergent signaling cascades from G protein–coupled receptors (*43*), such as NK1, EP, or mGlu receptors, and receptor tyrosine kinases, such as the trkB receptor, all of which are present in the superficial dorsal

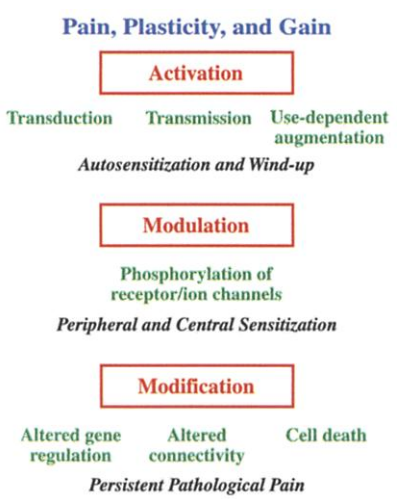

Fig. 1. The three forms of neural plasticity that increase gain in the somatosensory system to produce pain hypersensitivity are illustrated, highlighting changes they produce and their effects on pain transmission.

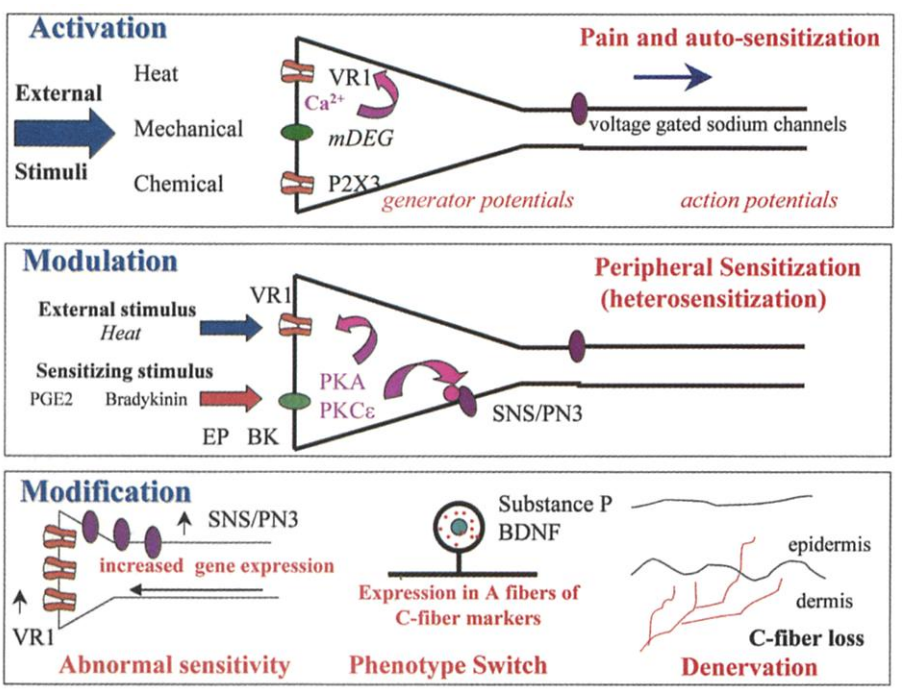

Fig. 2. Neural plasticity in primary sensory neurons. Activation, modulation, and modification all alter the nociceptor peripheral terminal threshold.

horn where nociceptors terminate (*44*). A likely point of convergence for G protein–coupled receptor and receptor tyrosine kinase signaling in the pathway to enhanced NMDA receptor function is PKC, which has been shown in hippocampal neurons to potentiate NMDA currents indirectly through activating Src (*43*).

Another kinase signaling cascade involved in synaptic plasticity is the mitogen-activated protein kinase pathway (*33*). In the superficial dorsal horn, MAPK phosphorylation increases following nociceptive stimulation and inhibiting it suppresses the second phase of the formalin test (*45*), which has been thought, because of its selective sensitivity to intrathecal NMDA receptor antagonists, (*46*) to be indicative of a suppression of central sensitization.

NMDA receptor-independent mechanisms for facilitating excitatory synaptic transmission are also potentially important in the pain system. In particular, subpopulations of dorsal horn neurons express AMPA receptors lacking the edited form of the GluR2 subunit. These AMPA receptors allow calcium influx sufficient to produce lasting facilitation of synaptic transmission in dorsal horn neurons (*47*).

Long-lasting depression of inhibition: Disinhibition. Also important for central sensitization is depression of spinal inhibitory mechanisms. Long-term depression of transmission at primary afferent synapses onto substantia gelatinosa neurons, many of which are GABA/glycinergic, can be elicited by activation of Aδ primary afferents (*48*). The depression, which requires NMDA receptor activation and a postsynaptic calcium increase, is likely to be mechanistically similar to LTD in other brain regions.

Modification of the Pain System

Modification of primary sensory neurons. Target-derived growth factors retrogradely transported from the target to the cell body are essential for survival during development, but have a role in the maintenance of neuronal phenotype in the adult (*49*). Increases in these signal molecules, which occur after inflammation, and decreases after loss of contact with the target after peripheral axon damage, initiate marked alterations in the expression of transmitters, synaptic neuromodulators, ion channels, G protein–coupled receptors, and growth-associated and structural proteins. Apart from retrograde signals, electrical activity alone, due to calcium influx through voltage-gated ion channels, can change transcription in sensory neurons (*44*).

After inflammation, there is an up-regulation of VR1 and SNS (*50, 51*), which, when transported to the peripheral terminal, may increase sensitivity to inflammatory mediators and susceptibility to peripheral sensitization. Increases in substance P and brain-derived neurotrophic factor (BDNF), two synaptic neuromodulators, also alter the central drive evoked in the dorsal horn by nociceptors (*52–54*).

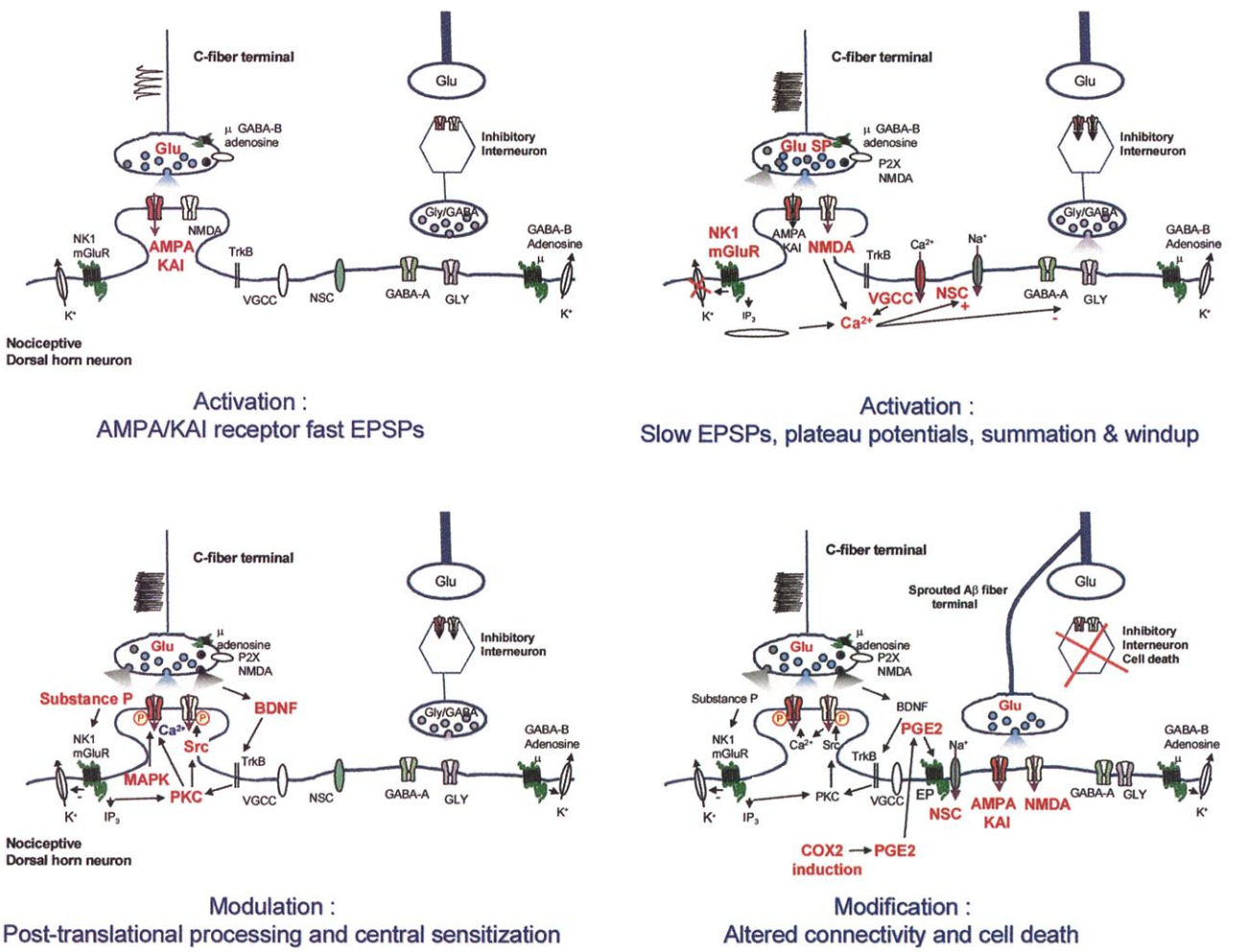

Fig. 3. Modes of neural plasticity at synapses onto nociceptive transmission neurons in the dorsal horn of the spinal cord. The neurons are activated by fast EPSPs and enhanced by slow EPSPs, plateau potentials, and windup. Modulation through intracellular kinase/phosphatase signaling cascades produces central sensitization through facilitating AMPA/kainate and NMDA receptor function or cell-surface expression. Modification is mediated by induced expression of gene products, loss of inhibitory interneurons, and establishment of aberrant excitatory synaptic connections.

Although modification involves an increase in constitutively expressed genes, an important feature also is the induction of novel genes. A-fiber neurons begin to express substance P and BDNF after inflammation (*44, 55*), and this phenotype shift contributes to the capacity of tactile stimuli applied to inflamed tissue to induce central sensitization (*56*).

Peripheral nerve injury generally produces changes in sensory neurons that are the opposite of those that occur after inflammation, with decreases in substance P, CGRP, VR1, and SNS/PN3 (*51, 57*). Some changes are common to both, such as an increase in BDNF (*58*), but some are unique to axotomy: an increase in the brain III sodium channel and a decrease in the μ opiate receptor (MOR) (*59, 60*). Brain III may mediate ectopic activity in injured sensory neurons, while decreases in MOR may contribute to diminished opiate sensitivity. Interestingly, peripheral nerve injury induces phenotypic shifts similar to inflammation, with expression of substance P and BDNF in A-fibers (*61, 62*) that may enable these fibers to evoke central sensitization.

Nerve injury results in a delayed loss of sensory neurons, an effect that is more prominent for C- than for A-fiber neurons (*63*). In addition, there is a central reorganization of A-fibers, which sprout from their deep dorsal horn laminar location up into that part of the spinal cord where C-fibers normally terminate and make functional synaptic contacts (*64–66*). The resultant changed connectivity might be a factor in the intractable nature of many neuropathic pains.

Modification of pain transmission neurons. Both inflammation and nerve injury induce transcriptional changes in dorsal horn neurons, which may be mediated by activation of the MAPK/pCREB cascade. These include changes in receptors (NK1, TrkB, GABA-R), and transmitters (dynorphin, enkephalin, GABA), and the induction of COX2 in dorsal horn neurons (*67–69*).

Two general patterns of central modification appear prominent: (i) an increase after peripheral inflammation of the expression of receptors in dorsal horn neurons for transmitters increased in primary sensory neurons, priming the system to produce increased modulation (*49*), and (ii) a reduction in inhibition after nerve injury. The latter may occur by a reduction in transmitters (*70*) or receptors (*71*) or a loss of inhibitory interneurons. Nerve injury, by virtue of injury discharge and ectopic activity, appears to lead to a cell death in the superficial laminae of the dorsal horn (*72, 73*), where inhibitory interneurons are concentrated, and by producing disinhibition may facilitate pain transmission.

Conclusion

Pain hypersensitivity is an expression of neuronal plasticity, the duration of which is determined by the particular patterns of activation, modulation, or modification occurring. These forms of plasticity constitute a continuum encompassing the diverse reactions of neurons to changes in their activity or environment. Pain is not a passive consequence of the transfer of a defined peripheral input to a pain center in the cortex, but an active process generated partly in the periphery and partly within the CNS by multiple plastic changes that together determine the gain of the system. The understanding of plasticity is rapidly increasing, and we expect that the future will provide further exciting insights into pain mechanisms.

References

1. E. W. McCleskey and M. S. Gold, *Annu. Rev. Physiol.* **61**, 835 (1999).
2. M. J. Caterina, T. A. Rosen, M. Tominaga, A. J. Brake, D. Julius, *Nature* **398**, 436 (1999).
3. R. Waldmann and M. Lazdunski, *Curr. Opin. Neurobiol.* **8**, 418 (1998).
4. P. Li *et al.*, *Nature* **397**, 161 (1999).
5. N. Chery and Y. De Koninck, *J. Neurosci.* **19**, 7342 (1999).
6. M. J. Caterina *et al.*, *Nature* **389**, 816 (1997).
7. D. Andrew and J. D. Greenspan, *J. Neurophysiol.* **82**, 2649 (1999).
8. S. Guenther, P. W. Reeh, M. Kress, *Eur. J. Neurosci.* **11**, 3143 (1999).
9. A. W. Duggan, P. J. Hope, B. Jarrott, H.-G. Schaible, S. M. Fleetwood-Walker, *Neuroscience* **5**, 195 (1990).
10. S. W. N. Thompson, A. E. King, C. J. Woolf, *Eur. J. Neurosci.* **2**, 638 (1990).
11. L. G. Sivilotti, S. W. N. Thompson, C. J. Woolf, *J. Neurophysiol.* **69**, 1621 (1993).
12. M. L. Mayer, G. L. Westbrook, P. B. Guthrie, *Nature* **309**, 261 (1984).
13. V. Morisett and F. Nagy, *J. Neurosci.* **19**, 7309 (1999).
14. C. J. Woolf and R. J. Mannion, *Lancet* **353**, 1959 (1999).
15. X. Shu and L. M. Mendell, *Neurosci. Lett.* **274**, 159 (1999).
16. P. W. Reeh, *Cellular Mechanisms of Sensory Processing*, NATO ASI series, *H: Cell Biology*, vol. 79, L. Urban, Ed. (Springer-Verlag, Berlin, 1994), pp. 119–131.
17. K. Mizimura and T. Kumazawa, *The Polymodal Receptor: A Gateway to Pathological Pain*, T. Kumazawa, L. Kruger, K. Mizimura, Eds. (Elsevier, Amsterdam, 1996), pp. 115–141.
18. K. O. Aley and J. D. Levine, *J. Neurosci.* **19**, 22181 (1999).
19. S. G. Khasar, G. McCarter, J. D. Levine, *J. Neurophysiol.* **81**, 1104 (1999).
20. P. Cesare, L. V. Dekker, A. Sardini, P. J. Parker, P. McNaughton, *Neuron* **23**, 617 (1999).
21. E. M. Fitzgerald, K. Okuse, J. N. Wood, A. C. Dolphin, S. J. Moss, *J. Physiol. (London)* **516**, 433 (1999).
22. M. S. Gold, D. B. Reichling, M. J. Schuster, J. D. Levine, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 1108 (1996).
23. S. England, S. Bevan, R. J. Docherty, *J. Physiol. (London)* **495**, 429 (1996).
24. S. G. Khasar, M. S. Gold, J. D. Levine, *Neurosci. Lett.* **256**, 17 (1998).
25. A. N. Akopian *et al.*, *Nature Neurosci.* **2**, 541 (1999).
26. C. J. Woolf, *Nature* **306**, 686 (1983).
27. M. Koltzenburg, L. K. Wahren, H. E. Torebjork, *Pfluegers Arch.* **420**, R52 (1992).
28. C. J. Woolf and A. E. King, *J. Neurosci.* **10**, 2717 (1990).
29. D. A. Simone *et al.*, *J. Neurophysiol.* **66**, 228 (1991).
30. Z. Ali, R. A. Meyer, J. N. Campbell, *Pain* **68**, 401 (1996).
31. S. Kilo, M. Schmelz, M. Koltzenburg, H. O. Handwerker, *Brain* **117**, 385 (1994).
32. M. Randic, M. C. Jiang, R. Cerne, *J. Neurosci.* **13**, 5228 (1993).
33. R. C. Malenka and R. A. Nicoll, *Science* **285**, 1870 (1999).
34. T. R. Soderling and V. A. Derkach, *Trends Neurosci.* **23**, 75 (2000).
35. M. W. Salter, *Biochem. Pharmacol.* **56**, 789 (1998).
36. X. M. Yu, R. Askalan, G. J. Keil, M. W. Salter, *Science* **275**, 674 (1997).
37. P. Li *et al.*, *Nature Neurosci.* **2**, 972 (1999).
38. C. J. Woolf, P. Shortland, L. G. Sivilotti, *Pain* **58**, 141 (1994).
39. D. A. Simone, T. K. Baumann, J. G. Collins, R. H. LaMotte, *Brain Res.* **486**, 185 (1989).
40. J. N. Campbell, S. N. Raja, R. A. Meyer, S. E. McKinnon, *Pain* **32**, 89 (1988).
41. A. Stubhaug, H. Breivik, P. K. Eide, M. Kreunen, A. Foss, *Acta Anaesthesiol. Scand.* **41**, 1124 (1997).
42. S. W. N. Thompson, C. J. Woolf, L. G. Sivilotti, *J. Neurophysiol.* **69**, 2116 (1993).
43. W. Y. Lu *et al.*, *Nature Neurosci.* **2**, 331 (1999).
44. R. J. Mannion *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 9385 (1999).
45. R. R. Ji, H. Baba, G. J. Brenner, C. J. Woolf, *Nature Neurosci.* **2**, 1114 (1999).
46. T. Yamamoto and T. L. Yaksh, *Anesthesiology* **77**, 757 (1992).
47. J. G. Gu, C. Albuquerque, C. J. Lee, A. B. MacDermott, *Nature* **381**, 793 (1996).
48. J. Sandkuhler, J. G. Chen, G. Cheng, M. Randic, *J. Neurosci.* **17**, 6483 (1997).
49. C. J. Woolf and M. Costigan, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7723 (1999).
50. G. J. Michael and J. V. Priestley, *J. Neurosci.* **19**, 1844 (1999).
51. S. Tate *et al.*, *Nature Neurosci.* **1**, 653 (1998).
52. Q.-P. Ma and C. J. Woolf, *J. Physiol. (London)* **486**, 769 (1995).
53. R. J. Traub, *Pain* **67**, 151 (1996).
54. B. J. Kerr *et al.*, *J. Neurosci.* **19**, 5138 (1999).
55. S. Neumann, T. P. Doubell, T. A. Leslie, C. J. Woolf, *Nature* **384**, 360 (1996).
56. Q.-P. Ma and C. J. Woolf, *Pain* **67**, 97 (1996).
57. T. Hokfelt, X. Zhang, Z. Wiesenfeld-Hallin, *Trends Neurosci.* **17**, 22 (1994).
58. G. J. Michael, S. Averill, P. Shortland, Q. Yan, J. V. Priestley, *Eur. J. Neurosci.* **11**, 3539 (1999).
59. J. A. Black *et al.*, *J. Neurophysiol.* **82**, 2776 (1999).
60. J. F. deGroot, R. E. Coggeshall, S. M. Carlton, *Neurosci. Lett.* **233**, 113 (1997).
61. K. Noguchi, Y. Kawai, T. Fukuoka, E. Senba, K. Miki, *J. Neurosci.* **15**, 7633 (1995).
62. X. F. Zhou *et al.*, *Neuroscience* **92**, 841 (1999).
63. R. E. Coggeshall, H. A. Lekan, T. P. Doubell, A. Allchorne, C. J. Woolf, *Neuroscience* **77**, 1115 (1997).
64. C. J. Woolf, P. Shortland, R. E. Coggeshall, *Nature* **355**, 75 (1992).
65. H. R. Koerber, K. Mirnics, A. M. Kavookjian, A. R. Light, *J. Neurophysiol.* **81**, 1636 (1999).
66. I. Kohama, K. Ishikawa, J. D. Kocsis, *J. Neurosci.* **20**, 1538 (2000).
67. K. Noguchi, R. Dubner, M. A. Ruda, *Neuroscience* **46**, 561 (1992).
68. K. E. McCarson and J. E. Krause, *J. Neurosci.* **14**, 712 (1994).
69. C. Hay and J. S. de Belleroche, *Neuroreport* **8**, 1249 (1997).
70. J. M. Castro-Lopes, I. Tavares, A. Coimbra, *Brain Res.* **620**, 287 (1993).
71. T. Fukuoka *et al.*, *Pain* **78**, 13 (1998).
72. T. Sugimoto, G. J. Bennett, K. C. Kajander, *Pain* **42**, 205 (1990).
73. J. J. Azkue, M. Zimmermann, T. F. Hsieh, T. Herdegen, *Eur. J. Neurosci.* **10**, 2204 (1998).