Proc. Natl. Acad. Sci. USA
Vol. 96, pp. 7693–7696, July 1999
Colloquium Paper

This paper was presented at the National Academy of Sciences colloquium "The Neurobiology of Pain," held December 11–13, 1998, at the Arnold and Mabel Beckman Center in Irvine, CA.

Neurotrophins and hyperalgesia

X.-Q. SHU AND L. M. MENDELL*

Department of Neurobiology and Behavior, State University of New York, Stony Brook, NY 11794

ABSTRACT **Nerve growth factor (NGF), a member of the neurotrophin family, is crucial for survival of nociceptive neurons during development. Recently, it has been shown to play an important role in nociceptive function in adults. NGF is up-regulated after inflammatory injury of the skin. Administration of exogenous NGF either systemically or in the skin causes thermal hyperalgesia within minutes. Mast cells are considered important components in the action of NGF, because prior degranulation abolishes the early NGF-induced component of hyperalgesia. Substances degranulated by mast cells include serotonin, histamine, and NGF. Blockade of histamine receptors does not prevent NGF-induced hyperalgesia. The effects of blocking serotonin receptors are complex and cannot be interpretable uniquely as NGF losing its ability to induce hyperalgesia. To determine whether NGF has a direct effect on dorsal root ganglion neurons, we have begun to investigate the acute effects of NGF on capsaicin responses of small-diameter dorsal root ganglion cells in culture. NGF acutely conditions the response to capsaicin, suggesting that NGF may be important in sensitizing the response of sensory neurons to heat (a process that is thought to operate via the capsaicin receptor VR1). We also have found that ligands for the trkB receptor (brain-derived neurotrophic factor and neurotrophin-4/5) acutely sensitize nociceptive afferents and elicit hyperalgesia. Because brain-derived neurotrophic factor is up-regulated in trkA positive cells after inflammatory injury and is transported anterogradely, we consider it to be a potentially important peripheral component involved in neurotrophin-induced hyperalgesia.**

It is now well established that, in late embryonic life, sensory neurons depend on the availability of peripherally derived factors for survival; this dependence is referred to as the neurotrophic hypothesis (1). Nociceptive neurons require nerve growth factor (NGF), which is a member of the neurotrophin family; the other members are brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and NT-4/5 (2). Neurotrophins signal via two types of receptors, the high-affinity trk receptor and the low-affinity p75 receptor (3). The high-affinity receptor for NGF is trkA; for BDNF and NT-4/5, it is trkB; for NT-3, it is trkC. The p75 receptor can be activated by all members of the neurotrophin family (2, 3).

Although neurotrophins have been generally considered to function during embryonic life, it is now clear that their importance continues well beyond this period. For example, nociceptors do not depend on NGF for survival beyond postnatal day 2, but they require the availability of NGF to maintain their phenotype during a postnatal critical period (4). trk receptors continue to be expressed on sensory neurons in adults (5), and neurotrophins also continue to be synthesized by numerous cell types (6).

NGF and Thermal Hyperalgesia. In recent years, it has become clear that NGF plays an important role in the function of nociceptive afferents in the adult (7). Specifically, the continued presence of trkA receptors on nociceptive afferent fibers (8) and the up-regulation of its ligand NGF in the skin during inflammation (9) indicate a potential role for NGF in inflammatory pain. Confirmation of a hyperalgesic action for NGF has been obtained by demonstrating that administration of NGF produces thermal and mechanical hyperalgesia (10). The thermal hyperalgesia has its onset within 1 h of NGF application whether it is systemic (10) or local (11). This short latency and the effectiveness of local peripheral administration suggest a peripheral mechanism underlying the hyperalgesia. Mechanical hyperalgesia typically has a latency of several hours, indicating a more complex mechanism, probably involving central processes (12). There is also evidence for independent, longer-latency, peripheral (13) and central (12) mechanisms underlying thermal hyperalgesia. The following discussion is restricted to peripheral mechanisms of neurotrophin-induced hyperalgesia.

Two types of evidence have been obtained in support of a peripheral locus for the short-latency thermal hyperalgesia initiated by NGF. Pharmacological studies indicate a role for peripherally located nonneural cells in NGF-induced hyperalgesia. The cell most centrally implicated in this action is the mast cell. These immunocompetent cells express trkA receptors (14) and degranulate their contents in response to NGF stimulation (15). These contents include serotonin (5-HT), histamine, and NGF itself (16). Lewin *et al.* (12) showed that prior degranulation of mast cells with compound 48/80 prevented the short-latency hyperalgesic effects of NGF without affecting long-latency ones. Systemic application of receptor blockers for 5-HT blocked the hyperalgesic effect of 5-HT. Because 5-HT has been shown, under some conditions, to sensitize the response of polymodal nociceptors to noxious heat (17, 18), the conclusion was that NGF was acting to sensitize primary afferent fibers via the release of 5-HT from mast cells. However, a more careful consideration of this effect indicates that the 5-HT receptor blockers had a paradoxical effect of converting the action of NGF from hyperalgesia to hypoalgesia (12). This effect raises the possibility that these blockers are acting to disturb some balance of effects initiated by NGF rather than simply blocking the sensitizing action of mast-cell 5-HT. Recently, blockers of the 5-HT_2 receptor and the histamine H_1 receptor were shown to be ineffective in blocking the effect of NGF-induced hyperalgesia (19). Together, these findings suggest that other mast-cell contents (e.g., NGF) may play a more important role than 5-HT or histamine in NGF-induced hyperalgesia (see below).

A more direct approach toward demonstrating a peripheral component to NGF-induced hyperalgesia was adopted by Rueff and Mendell (20), who examined the response of

Abbreviations: NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT-*n*, neurotrophin-*n*; 5-HT, serotonin; DRG, dorsal root ganglion.

*To whom reprint requests should be addressed. e-mail: lmendell@neurobio.sunysb.edu.

PNAS is available online at www.pnas.org.

small-diameter nociceptive afferents in an isolated skin-nerve preparation to NGF applied directly to the receptive field. Individual small-diameter afferent fibers whose conduction velocity was established by the latency of their response to electrical stimulation in the receptive field were selected if they responded to high-intensity mechanical stimulation. The response to thermal stimulation was established. NGF then was applied directly to the receptive field for 20 min, and the mechanical and thermal responses were determined again. NGF was found to lower the threshold to thermal stimulation by about 2°C, a change that was statistically significant. However, no change was noted in the mechanical threshold. Application of saline was found to elicit no effect. In later experiments, NT-3 applied to the receptive field also was determined not to alter the threshold to noxious heat (11). If the animals were pretreated with compound 48/80, NGF had no effect on the noxious heat threshold of individual nociceptive afferents. These experiments established that NGF changes the threshold of nociceptive afferents and that mast cells are involved. They also confirmed a peripheral locus for the sensitizing action of NGF.

The experiments described so far show that NGF is *sufficient* to elicit hyperalgesia. A crucial question is whether it is a *necessary* intermediate. This issue has been explored by preventing the increase in NGF after an inflammatory injury induced experimentally by agents such as complete Freund's adjuvant. Antibodies to NGF (12, 21) or the immunoadhesin molecule trkA-IgG (22) were used to prevent the increase of NGF levels. A uniform finding in these experiments was that hyperalgesia was abolished, suggesting that NGF is a necessary intermediate in inflammatory hyperalgesia induced by molecules such as complete Freund's adjuvant. It is presently believed that injury leads to release of cytokines, such as tumor necrosis factor-α and IL-1β, which cause the release of NGF from cells such as keratinocytes and fibroblasts (23). Such a release would initiate the degranulation of mast cells as illustrated in Fig. 1.

Direct Effects of NGF on Nociceptors. Although the experiments described thus far indicate an indirect role for NGF in peripheral sensitization via mast cells, the exact locus of action for NGF is not fixed by these findings. The reason for this uncertainty is that mast cells contain NGF, which would be released on degranulation. Thus, activation of mast cells by NGF might lead to the release of more NGF, and the presence of trkA receptors on sensory afferents could provide the ability for a direct effect on the peripheral threshold. Furthermore, repeated daily administration of NGF can eventually produce hyperalgesia despite maintained mast-cell degranulation (24), suggesting that, under some circumstances, mast cells can be bypassed. It is already known that NGF can affect the function of sensory neurons directly, because, both in culture (25) and *in vivo* (21), exogenous NGF administration leads to up-regulation of peptides such as substance P and calcitonin gene-related peptide in the cell body. However, this up-regulation is a relatively slow effect (hours to days) involving transcriptional mechanisms and would be much too slow to account for the rapid effect of NGF on sensory thresholds.

We have initiated experiments to examine whether NGF can rapidly increase the threshold of sensory neurons directly. To accomplish this increase, it is necessary to provide a stimulus that excites these neurons and to determine whether NGF sensitizes the response. To determine whether this effect is direct, these experiments must be done in culture to avoid the potential actions of other cells such as mast cells.

Both behavioral evidence and electrophysiological evidence indicate that peripheral nociceptors are very sensitive to capsaicin (26, 27), an ingredient in hot peppers. When capsaicin is applied directly to a cell, it is depolarized as a consequence of a nonspecific increase in permeability to cations including Ca. Recently, the capsaicin receptor was cloned (28). This receptor, named VR1, when expressed in oocytes, is also sensitive to noxious heat, suggesting that the noxious-heat response of primary afferents is mediated via the VR1 receptor. However, the component of the VR1 receptor sensitive to heat and to capsaicin may be different. NGF has been shown to play some role in the expression of these receptors, because dorsal root ganglion (DRG) cells cultured for several days in the absence of NGF fail to display capsaicin sensitivity (29).

Recently, we have shown that capsaicin responses are influenced acutely by NGF (X.-Q.S. and L.M.M., unpublished work). Capsaicin was pressure ejected on the somata of small dissociated (<30 μm) DRG neurons that were recorded in whole-cell or perforated patch clamp. With the cell voltage clamped at −60 mV, an inward current was observed. A second identical capsaicin pulse 10 min later resulted uniformly in a substantially smaller current (Figs. 2 and 3). We found that bath-applying NGF during the 10-min interval (Fig. 2) often resulted in elimination of the tachyphylaxis and that the second response was often larger than the first (Fig. 3), as much as twice as large. In some cells, tachyphylaxis after NGF

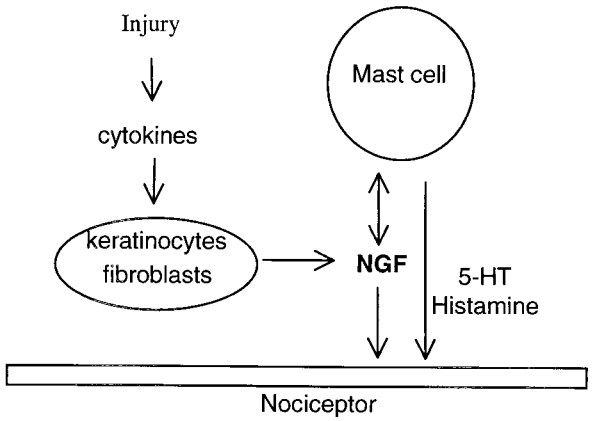

FIG. 1. Schematic diagram outlining the relationship of mast cells, nociceptors, and NGF as well as how this system is activated as a consequence of peripheral injury. Skin injury leads to release of cytokines, such as tumor necrosis factor-α and IL-1β, which activate cells, such as keratinocytes and fibroblasts, to release NGF. The NGF can activate nociceptors directly but, in addition, can cause mast cells to degranulate their products, including 5-HT, histamine, and NGF. This endogenous source of NGF seems to be more potent than exogenous NGF in sensitizing nociceptors (see text for further details).

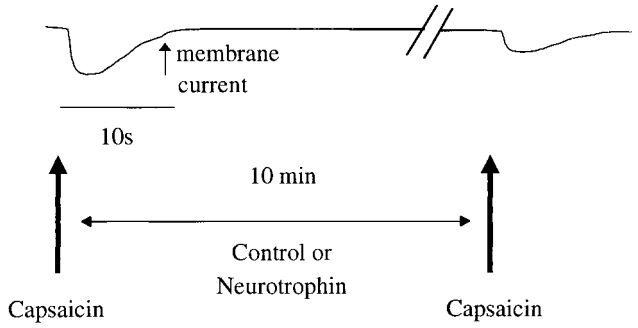

FIG. 2. Protocol for testing NGF effects on capsaicin currents on DRG cells recorded in perforated patch clamp. Cells chosen for this analysis were <30 μm in diameter. A microelectrode filled with 1 μM capsaicin was placed close to the patched cell, and the capsaicin was ejected via a brief (400-ms) pressure pulse. The cell was clamped at −60 mV, and the inward capsaicin current lasting ≈10 s is shown at the top. A second pressure pulse was delivered 10 min later. During the interval, the cell was exposed either to control saline solution or to 100 ng/ml of NGF. In controls, the response to the second capsaicin pulse was always smaller than the initial one.

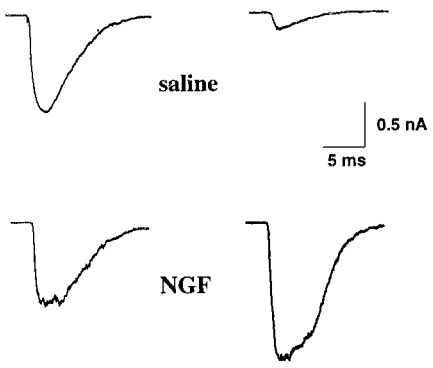

FIG. 3. Examples of capsaicin responses in two DRG cells, the first conditioned by a 10-min exposure to saline (*Upper*) and the second conditioned by 100 ng/ml NGF (*Lower*). Note the smaller response to the second capsaicin pulse (tachyphylaxis) after saline treatment and the larger response when NGF is placed in the medium.

treatment was similar to that observed after saline treatment. We assume that these cells do not express trkA.

If we assume that the heat response of nociceptors is mediated by peripheral capsaicin receptors (28), it is apparent that the sensitizing effect of NGF may be direct on the capsaicin receptor. It might involve phosphorylation of the capsaicin receptor that is dephosphorylated as a consequence of the initial capsaicin stimulus (30). However, the assumption that the heat response is sensitized in the same way as the response to capsaicin requires direct proof.

These experiments were carried out on DRG cell bodies maintained in culture for up to 1 day. The question that inevitably arises is whether these conditions adequately test what might be occurring in the terminals. One problem is the presence of the nucleus in the cultured DRG cells, which would not be present in the terminals *in vivo*. However, the NGF effect on the capsaicin response in the present experiments was very rapid, within 10 minutes, which is too fast to require transcription. It follows that the influence of NGF on the capsaicin response does not involve transcription and probably occurs at a membrane and/or cytoplasmic level.

The finding that NGF directly affects the capsaicin response of DRG cells suggests that NGF should be able to directly sensitize the response to noxious heat. If so, why should prior degranulation of mast cells be able to abolish the hyperalgesic effects of NGF? In other words, why should mast cells be necessary for NGF-induced hyperalgesia if NGF can directly sensitize nociceptive neurons as shown in Fig. 3? One possibility is that exogenous NGF is insufficient in amount or does not reach the terminal in large enough concentrations in the intact skin to influence nociceptive endings *in vivo*. However, if exogenous NGF degranulates mast cells, and these, in turn, release NGF, then the resulting positive feedback cycle would amplify the amount of NGF activating the sensory ending. In effect, this hypothesis suggests that exogenous NGF acts as a trigger that liberates NGF from mast cells. This latter source of NGF is postulated to be necessary because of its intimate relationship with nociceptive afferent terminals, which allows it to condition the nociceptive terminal. The second possibility is that other substances released from the mast cell, such as 5-HT or histamine, are necessary to enable NGF to have its full effect on nociceptive terminals.

trkB Agonists and Hyperalgesia. A recent finding of interest in the context of neurotrophin involvement in hyperalgesia is that administration of exogenous NGF leads to up-regulation of the trkB agonist BDNF in trkA-expressing sensory neurons (31, 32). There is evidence that BDNF can be transported anterogradely in intact axons to the periphery (33), raising the possibility that this neurotrophin plays some role in inflammation-triggered events. In support of this possibility, both trkB agonists (BDNF and NT-4) were found to evoke heat hyperalgesia when injected locally into the skin, and both of these agents were shown to elicit sensitization of individual nociceptive afferent fibers to noxious heat (11, 20). Both the hyperalgesia (11) and the acute fiber sensitization (20) elicited by NT-4 were blocked in mast-cell-depleted preparations, suggesting the operation of a mechanism similar to the one mediating the hyperalgesic effects of NGF. The interpretation of these findings is still uncertain, because it is not known whether skin mast cells express functional trkB receptors. Intraperitoneal mast cells are known to express only trkA receptors (14), but blood mast cells, for example, express trkA, trkB, and trkC receptors (34).

NT-3 did not elicit peripheral sensitization to noxious heat (11). This result might be interpreted as indicating that mast cells responsible for this effect do not express trkC receptors. However, the selectivity probably resides with the afferent fibers, because it is known that trkC receptors are expressed only on large-diameter afferents, whereas trkA is expressed only on small-diameter afferents (5). trkB is expressed on cells in the DRG with a wide range of sizes, with considerable numbers of cells coexpressing trkA and trkB. However, there is virtually no coexpression of trkA and trkC on sensory neurons (5).

CONCLUSIONS

If we consider capsaicin as a surrogate for noxious heat in activating the VR1 receptor (28), our findings suggest that NGF acts as a peripheral sensitizing agent, at least in part by sensitizing the response of the nociceptor to noxious heat directly. NGF is not the first such sensitizing agent to be described. It has been known that other sensitizing agents exist, including prostaglandins and bradykinin. Previously, prostaglandin E2 has been shown to sensitize sensory neurons to capsaicin (35). Acute exposure of cultured neonatal DRG cells to bradykinin can enhance their sensitivity to capsaicin and to low pH (36).

These agents do not seem to function in isolation. For example, bradykinin activates postganglionic efferents in the skin, and these release prostaglandin E2 (37). Inactivation of postganglionic efferents reduces NGF-induced hyperalgesia (38), indicating interaction between NGF and prostaglandins in hyperalgesia. NGF also interacts with bradykinin, in part by stimulating the delayed up-regulation of BK1 receptors via release of kallikreins from mast cells (see ref. 13 for review). Blockade of BK1 receptors can transiently diminish NGF-induced hyperalgesia (13), further indication of the interrelationship of these agents in causing peripheral sensitization.

Thus, NGF seems to be one of a number of sensitizing agents present in peripheral tissues. What is not clear at present is what the integrative function of these individual agents might be in causing pain associated with inflammatory injury. An answer might be found by considering the principal cellular events responsible for production of these different agents: cell breakdown for prostaglandin E2, clotting for bradykinin, and the immune reaction (mast cells) for NGF (see ref. 39 for review). Evidently, the hyperalgesia accompanying inflammation is sufficiently adaptive in terms of protection by immobilization of the affected body part that redundant mechanisms involving mast cells (12), sympathetic efferents (38), and neutrophils (19) have evolved. However, in some cases hyperalgesia becomes maladaptive, particularly if it elicits central sensitization (40) that causes the hyperalgesia to outlast the peripheral damage. It then becomes essential to minimize peripheral sensitization to reduce both the immediate nociceptive effects as well as the long-lasting ones produced by central sensitization (40).

This work was supported by National Institutes of Health Grants NS-14899 and NS-32264. Partial support was furnished by the Javits Neuroscience Award NS-16996 (to L.M.M.). We thank Genentech for the NGF and NT-4/5 and Regeneron Pharmaceuticals (Tarrytown, NY) for the BDNF and NT-3 used in our studies reviewed in this paper.

1. Davies, A. M. (1996) *Philos. Trans. R. Soc. London B* **351,** 389–394.
2. Thoenen, H. (1991) *Trends Neurosci.* **14,** 165–170.
3. Barbacid, M. (1994) *J. Neurobiol.* **2,** 1386–1403.
4. Lewin, G. R., Ritter, A. M. & Mendell, L. M. (1992) *J. Neurosci.* **12,** 1896–1905.
5. McMahon, S. B., Armanini, M. P., Ling, L. H. & Phillips, H. S. (1994) *Neuron* **12,** 1161–1171.
6. Lindsay, R. M. (1996) *Philos. Trans. R. Soc. London B* **351,** 365–373.
7. Lewin, G. R. & Mendell, L. M. (1993) *Trends Neurosci.* **16,** 353–359.
8. Averill, S., McMahon, S. B., Clary, D. O., Reichardt, L. F. & Priestley, J. V. (1995) *Eur. J. Neurosci.* **7,** 1484–1494.
9. Weskamp, G. & Otten, U. (1987) *J. Neurochem.* **48,** 1779–1786.
10. Lewin, G. R., Ritter, A. M. & Mendell, L. M. (1993) *J. Neurosci.* **13,** 2136–2148.
11. Shu, X. Q., Llinas, A. & Mendell, L. M. (1999) *Pain* **80,** 463–470.
12. Lewin, G. M., Rueff, A. & Mendell, L. M. (1994) *Eur. J. Neurosci.* **6,** 1903–1912.
13. Rueff, A., Dawson, A. J. & Mendell L. M. (1996) *Pain* **66,** 359–372.
14. Horigome, K., Pryor, J. C., Bullock, E. D. & Johnson, E. M., Jr. (1993) *J. Biol. Chem.* **268,** 14881–14887.
15. Mazurek, N., Weskamp, G., Erne, P. & Otten, U. (1986) *FEBS Lett.* **198,** 315–320.
16. Leon, A., Buriani, A., Dal Toso, R., Fabris, M., Romanello, S., Aloe, L. & Levi-Montalcini, R. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3739–3743.
17. Beck, P. W. & Handwerker, H. O. (1974) *Pflügers Arch.* **347,** 209–222.
18. Rueff, A. & Dray, A. (1993) *Agents Actions* **38,** 13–15.
19. Bennett, G., al-Rasheed, S., Hoult, J. R. S. & Brain, S. D. (1998) *Pain* **77,** 315–322.
20. Rueff, A. & Mendell, L. M. (1996) *J. Neurophysiol.* **76,** 3593–3596.
21. Woolf, C. J., Safieh-Garabedian, B., Ma, Q.-P., Crilly, P. & Winter, J. (1994) *Neuroscience* **62,** 327–331.
22. McMahon, S. B., Bennett, D. L., Priestley, J. V. & Shelton, D. L. (1995) *Nat. Med.* **1,** 774–780.
23. Woolf, C. J., Allchorne, A., Safieh-Garabedian, B. & Poole, S. (1997) *Br. J. Pharmacol.* **121,** 417–424.
24. Amann, R., Schuligoi, R., Herzeg, G. & Donnerer, J. (1995) *Pain* **64,** 323–329.
25. Lindsay, R. M. & Harmar, A. J., (1989) *Nature (London)* **337,** 362–364.
26. LaMotte, R., Lundberg, L. E. & Torebjork, H. E. (1992) *J. Physiol. (London)* **448,** 749–764.
27. Szolcsanyi, J., Anton, F., Reeh, P. W. & Handwerker, H. O. (1988) *Brain Res.* **446,** 262–268.
28. Caterina, M. J., Schumacher, M. A., Tominaga, M., Rosen, T. A., Levine, J. D. & Julius D. (1997) *Nature (London)* **389,** 816–824.
29. Winter, J., Forbes, C. A., Sternberg, J. & Lindsay, R. M. (1988) *Neuron* **1,** 973–981.
30. Koplas, P. A., Rosenberg, R. L. & Oxford, G. S. (1997) *J. Neurosci.* **17,** 3525–3537.
31. Apfel, S. C., Wright, D. E., Wiideman, A. M., Dormia, C., Snider, W. D. & Kessler, J. A. (1996) *Mol. Cell. Neurosci.* **7,** 134–142.
32. Michael, G. J., Averill, S., Nitkunan, A., Rattray, M., Bennett, D. L. H., Yan, Q. & Priestley, J. V. (1997) *J. Neurosci.* **17,** 8476–8490.
33. Tonra, J. R., Curtis, R., Wong, V., Cliffer, K. D., Park, J. S., Timmes, A., Nguyen, T., Lindsay, R. M., Acheson, A. & DiStefano, P. S. (1998) *J. Neurosci.* **18,** 4374–4383.
34. Tam, S. Y., Tsai, M., Yamaguchi, M., Yano, K., Butterfield, J. H. & Galli, S. J. (1997) *Blood* **90,** 1807–1820.
35. Pitchford, S. & Levine, J. D. (1991) *Neurosci. Lett.* **132,** 105–108.
36. Stucky, C. L., Abrahams, L. G. & Seybold, V. S. (1998) *Neuroscience* **84,** 1257–1265.
37. Taiwo, Y. O., Heller, P. H. & Levine, J. D. (1990) *Neuroscience* **39,** 523–531.
38. Andreev, N. Y., Dimitrieva, N., Koltzenburg, M. & McMahon, S. B. (1995) *Pain* **63,** 109–115.
39. Levine, J. & Taiwo, Y. (1994) in *Textbook of Pain*, eds. Wall, P. D. & Melzack, R. (Churchill Livingstone, Edinburgh), pp. 45–56.
40. McMahon, S. B., Lewin, G. R. & Wall, P. D. (1993) *Curr. Opin. Neurobiol.* **3,** 602–610.