PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Neurotrophins and Nerve Injury in the Adult

V. M. K. Verge, K. A. Gratto, L. A. Karchewski and P. M. Richardson

Phil. Trans. R. Soc. Lond. B 1996 **351**, 423-430
doi: 10.1098/rstb.1996.0038

Neurotrophins and nerve injury in the adult

V. M. K. VERGE[1], K. A. GRATTO[1], L. A. KARCHEWSKI[1] AND P. M. RICHARDSON[2]

[1] *Department of Anatomy and Cell Biology and MS/Neuroscience Research Center, University of Saskatchewan, Saskatoon, Canada S7N 5E5*
[2] *Division of Neurosurgery, McGill University & The Montreal General Hospital Montreal, Canada H3G1A4*

SUMMARY

A role for neurotrophins in mature primary sensory neurons persists, extending beyond that of promoting survival during development, to one of maintaining phenotypic and functional properties. Many adaptive changes that occur after peripheral axotomy and in axonal repair are believed to be influenced by altered availability of neurotrophic molecules to the neuron in this state. Indeed, administration of exogenous nerve growth factor counteracts many degenerative changes observed in the subpopulation of axotomized neurons which are nerve growth factor-responsive. Current efforts focus on defining actions of other neurotrophins (brain-derived neurotrophic factor, neurotrophin-3 and neurotrophin-4/5) in nerve injury and repair, and the intracellular pathways involved. Knowledge gained from work focusing on nerve growth factor and neurotrophin-3 in supporting maintenance or modulation of aspects of the differentiated state of adult primary sensory neurons is discussed.

1. INTRODUCTION

Adult primary sensory neurons represent a diverse group; mirrored through differences in size, cytology, physiology, biochemical markers and responsiveness to neurotrophic factors (Hunt *et al.* 1992; Lawson 1992). Neurotrophins are a family of related proteins that individually serve as survival factors for largely distinct subclasses of developing DRG neurons and whose mammalian members include nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) (reviewed in Mendell 1995). They exert their effects via specific high-affinity binding to receptor tyrosine kinases known as trks; NGF interacting with trk (trkA), BDNF and NT-4/5 with trkB, and NT-3 with trkC (Kaplan *et al.* 1991; Klein *et al.* 1991; Lamballe *et al.* 1991; Ip *et al.* 1993). In addition to their interaction with trk receptors, each neurotrophin is capable of binding to a low-affinity neurotrophin receptor, p75. The exact importance of this dual receptor system is not known, however, it may serve to increase responsiveness of individual neurons to NGF (reviewed in Chao & Hempstead 1995) or selectively regulate retrograde neurotrophin transport (Curtis *et al.* 1995).

A number of well-documented experimental models have been developed to evaluate NGF's role in adult DRG. NGF appears to be important in maintaining phenotypic and functional properties associated with the intact axonal state (see papers by L. M. Mendell and G. R. Lewin, this volume), and re-establishing these properties after pathological insult. The precise role(s) for neurotrophins other than NGF in mature DRG are largely unknown. However, recent detailed analysis of deficits in sensory function resulting from

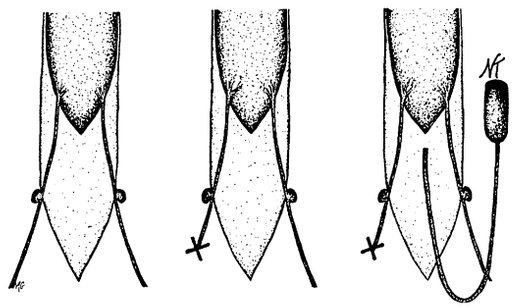


Figure 1. *In vivo* model employed to assess the role of neurotrophins in intact and injured sensory neurons. Left: DRG associated with intact sciatic nerves are used to characterize neurotrophin-responsive subpopulations. Middle: 2–3 week chronic unilateral sciatic nerve transection and resection at site of origin to the L4 & L5 spinal nerves is used to examine phenotypic cell body changes after injury. Right: intrathecal infusion of neurotrophin via mini osmotic pump for one week after a two week unilateral transection period is used to assess which of the injury-associated phenotypic changes or properties of intact neurons are altered in reponse to exogenous neurotrophin application. Note: frozen left and right DRG from injured and injured plus neurotrophin-infused animals are fused together and sectioned, allowing experimental and control groups to be processed under identical conditons on the same slide, thereby avoiding slide to slide variability resulting from histochemical procedures used (Verge *et al.* 1989*a*, *b*, 1990*a*, *b*; 1992, 1995; Gratto *et al.* 1994, 1995; Karchewski *et al.* 1995*a*, *b*).

homologous recombination experiments targeting neurotrophins or their receptors (Snider 1994; and see paper by Snider *et al.* this volume) and analysis of the distribution, co-localization and characterization of neurotrophin-responsive subpopulations has allowed for insights (Karchewski *et al.* 1995*a*; and see paper by H. S. Phillips *et al.* this volume), the most prominent being the requirement of NT-3 and trkC for proprioceptor development.

A long standing interest of our laboratories has been identifying and defining roles of molecules, principally neurotrophins, believed involved in nerve injury and repair of adult sensory neurons. A model was developed to test effects of individual neurotrophins on cellular properties of intact and injured DRG neurons (figure 1). The following discussion summarizes our and others' findings for NGF, and reports on recent studies examining NT-3's role.

2. NGF COUNTERACTS AXOTOMY-INDUCED CHANGES IN SENSORY NEURONS

After peripheral nerve insult, surviving DRG neurons undergo structural and biochemical changes including alterations in morphology, and expression of enzymes, peptides, growth-associated molecules, cytoskeletal proteins and receptors (reviewed in Aldskogius *et al.* 1992). They may lose their ability to respond to various stimuli or gain new reactivity. It is not known which factor(s) causes these alterations after axotomy, but it has been demonstrated that if regeneration is successful, many of the changes reverse (Lieberman 1974; Aldskogius *et al.* 1992). Several lines of evidence support the view that retrograde transport of trophic factor(s) expressed in limiting quantities in peripheral target and/or nonneuronal cells within the nerve help maintain the neuron in it's normal biological state (Goedert *et al.* 1981; Korsching & Thoenen 1983; Heumann *et al.* 1987; Funakoshi *et al.* 1993). For example, disruption of this flow, either by axotomy or application of microtubule formation inhibitors, triggers the injury-induced state, or aspects of it, in the neuron (Lieberman 1974; Fitzgerald *et al.* 1984; Kniyihar-Csillik *et al.* 1991; Kashiba *et al.* 1992). However, the most convincing evidence for the importance of retrograde neurotrophin(s) supply in the injury response, arises from experiments where either NGF is infused intrathecally at a time point after the neuron has responded to the injury (i.e. figure 1) or administered to the severed tip of the nerve. Using this approach trauma-induced transganglionic degenerative atrophy and decreases in NGF high-affinity bindings sites, trkA and p75; neuronal size; medium neurofilament subunit (NFM); peptides α- and β-calcitonin gene-related peptide (CGRP) and substance P (SP); flouride resistant acid phosphatase; and neuronal survival are partially prevented or reversed by exogenous NGF (Csillik *et al.* 1985; Fitzgerald *et al.* 1985; Rich *et al.* 1987; Verge *et al.* 1989*b*, 1990, 1992, 1995; Gold *et al.* 1991; Raivich *et al.* 1991; Inaishi *et al.* 1992). In contrast, addition of NGF mitigated, at least partially, trauma-induced increases in immediate early gene protein cJUN (Gold *et al.* 1993); and peptides galanin (GAL), neuropeptide tyrosine (NPY), vasoactive intestinal peptide (VIP) and cholecystokinin (CCK) (Verge *et al.* 1995). The ability of NGF to differentially regulate peptide expression (figure 2) and downregulate cJUN in injured sensory neurons, supports an involvement of NGF in suppression of cJUN and injury-associated peptides in the intact

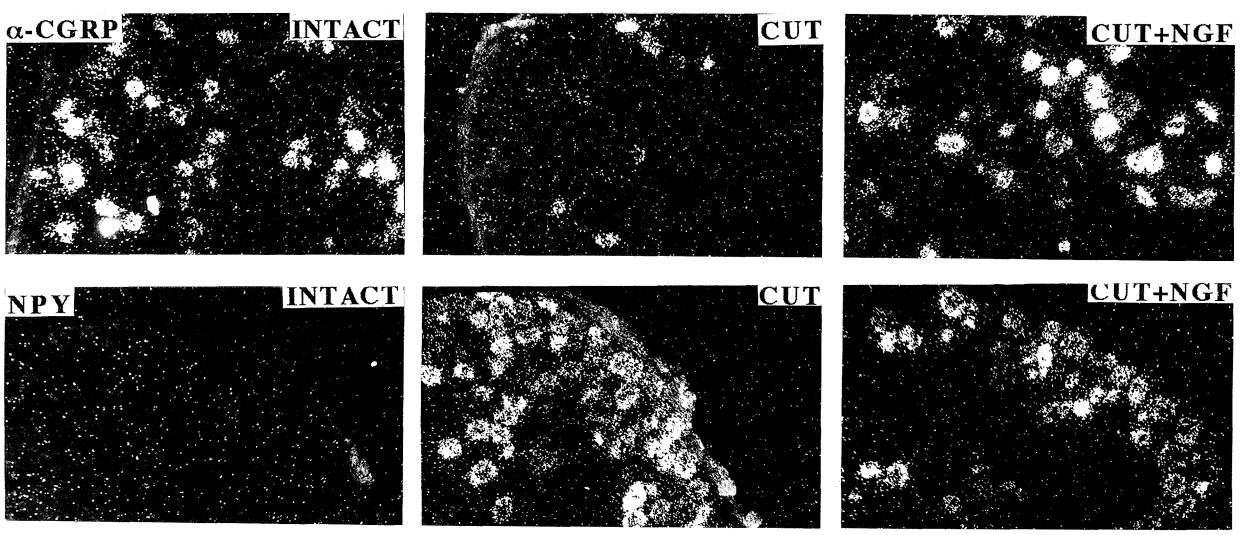


Figure 2. Differential influence of exogenous NGF on neuropeptide expression. Dark-field photomicrographs of L5 DRG sections (from the same animal series) processed for *in situ* hybridization using 35S-labelled oligonucleotide probes to detect α-CGRP (top row) and NPY (bottom row) mRNA and qualitatively demonstrate the ability of delayed intrathecal infusion of NGF (125–250 μg ml^{-1} h^{-1}) to injured neurons to both positively and negatively influence mRNA expression (Verge *et al.* 1995). In intact DRG many neurons express α-CGRP while few if any express NPY mRNA. Two weeks after injury, neuronal expression of α-CGRP is downregulated while NPY is heterogenously upregulated in injured neurons. Infusion of NGF for 7 days, 2 weeks after injury is effective in counteracting the axotomy-induced decrease in α-CGRP mRNA expression and mitigating the increase in NPY expression such that approximately one half fewer neurons now exhibit detectable hybridization signal for this peptide. Magnification × 57.

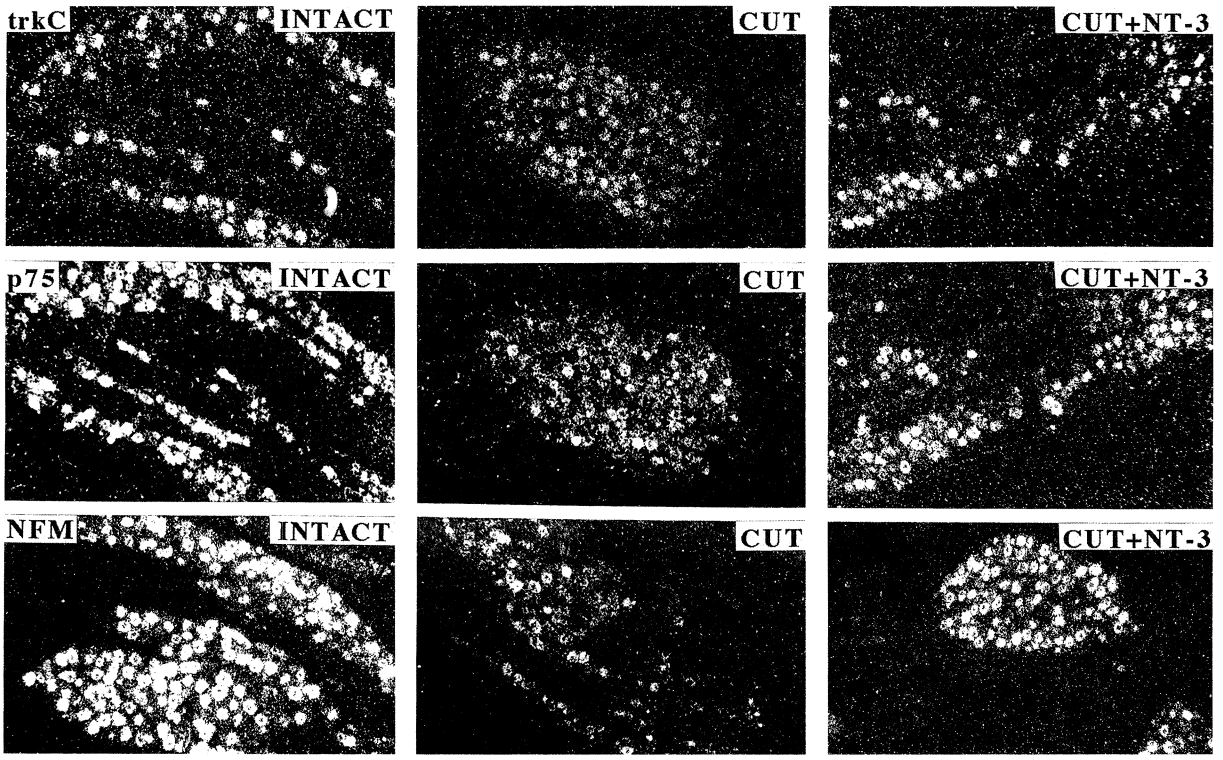


Figure 3. Ability of exogenous NT-3 to regulate NFM, p75 and trkC expression in injured lumbar sensory neurons (Gratto *et al.* 1994, 1995). Dark-field photomicrographs of 6 μm L5 DRG sections (all from the same animal series, p75 and trkC are adjacent sections) processed for *in situ* hybridization to detect mRNA as indicated in upper left of each row. In intact DRG NFM, p75 and trkC are abundantly and heterogenously expressed. Two weeks after injury, neuronal expression of all three messages is downregulated. Intrathecal infusion of NT-3 (600 ng μl^{-1} h^{-1}) for 7 days, 2 weeks after injury is effective in counteracting the axotomy-induced decrease in a subpopulation of the remaining injured neurons, quantitation of which is shown in figure 4. Magnification ×30.

state, with the post-trauma upregulation of these proteins being a consequence of inadequate supply of retrogradely transported NGF.

The capacity of NGF to counteract effects of axotomy appears selective for the subpopulation of NGF receptor-bearing neurons. In support, NGF does not appear to influence injury-induced decreases in expression of the peptide somatostatin (SOM), which was predicted, as somatostatinergic neurons neither display NGF high-affinity binding sites, nor express trkA mRNA (Verge *et al.* 1989*a*, 1995). A further example of the specificity of NGF's actions is the ability of NGF to modulate NFM and p75 mRNA levels after injury (Verge *et al.* 1990*a*, 1992). In normal DRG, expression of NFM mRNA is not correlated with NGF binding, but strongly correlated to neuronal volume. Thus, although approximately 15% of intact DRG neurons are medium to large in size, express abundant NFM mRNA and are NGF-responsive, there are an additional 25% which are also medium to large in size but do not display NGF high-affinity binding sites. Similarily, although a positive correlation exists between number of NGF high-affinity binding sites and expression of p75 mRNA in the 40% of DRG neurons which are NGF-responsive, many additional neurons, presumably responsive to another neurotrophin(s), also express moderate to high levels of p75. Sciatic nerve transection results in a dramatic downregulation of both p75 and NFM expression. This loss can be reversed by NGF infusion, but is apparent only in neurons displaying high-affinity NGF receptors and, therefore, presumably responsive to the ligand. These studies indicate that the expression of NGF high-affinity receptors, as well as unknown cell-specific factors allowing expression of the marker, are required for the NGF-rescue effect to be observed (Verge *et al.* 1990, 1992).

3. NT-3 COUNTERACTS AXOTOMY-INDUCED CHANGES IN SENSORY NEURONS

Axotomized neurons that are not NGF-responsive presumably respond, via their receptors, to other trophic factors. This hypothesis was recently tested for NT-3. *In situ* hybridization experiments indicate that 30–35% of intact adult DRG neurons strongly display hybridization signal for trkC, whereas another 10% express low levels of trkC mRNA (Karchewski *et al.* 1995*a*). Expression is detected in some small neurons, but primarily in medium to large sized neurons (McMahon *et al.* 1994; Karchewski *et al.* 1995*a*; Wetmore & Olson 1995; Wright & Snider 1995) believed to function in proprioception.

The influence of NT-3 on p75 and NFM mRNA concentrations in injured neurons can be demonstrated by delayed intrathecal infusion of NT-3, but is not readily apparent in uninjured neurons. This is because both markers are not only expressed at moderate to abundant levels in trkC-positive neurons, but are detectable in many additional neurons (figure 4).

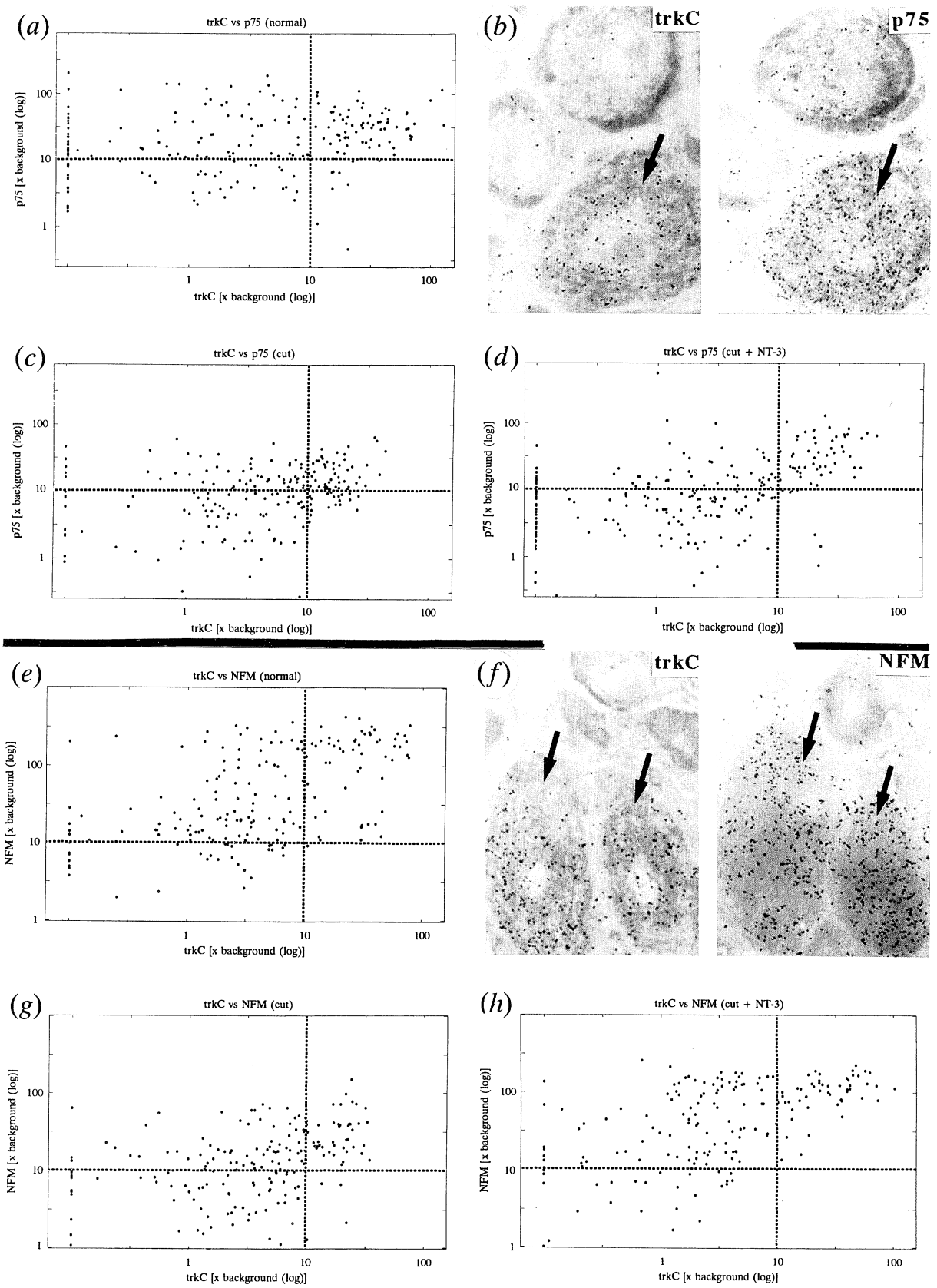


Figure 4. Quantification of data illustrated in figure 3. Scatterplots of labelling indices of 200–300 individual identified neurons in adjacent 6 μm thick sections of L5 DRG processed for either trkC (*x* axis, logarithmic scale), p75 (*y* axis, logarithmic scale) or NFM (*y* axis, logarithmic scale) *in situ* hybridization, to depict the relation between the two mRNAs examined in the upper (*a–d*) and lower series (*e–h*). Labelling index refers to the ratio of silver grain density over neuronal cytoplasm to grain density over areas of the neuropil devoid of positive hybridization signal. The left upper (*a*, *e*) and left lower (*c*, *g*) plots of each series pertain to left and right L5 DRG of a rat, with 3 week right sciatic nerve transection. The right lower plots of each series (*d*, *h*) describe the right L5 DRG of a rat with sciatic

Proximal sciatic nerve axotomy results in down-regulation of trkC, p75 and NFM mRNA expression as previously reported (Verge *et al.* 1990, 1992), which appears to be partially counteracted by delayed NT-3 infusion (Gratto *et al.* 1994, 1995; figure 3). Because NT-3 (at concentrations outside physiological ranges) has been shown *in vitro* to interact with trk receptors other than trkC (Soppet *et al.* 1991), it is important to determine whether p75 or NFM mRNA modulation by NT-3 occurs in neurons not expressing trkC. Figure 4 (quantitative analysis of data in figure 3) indicates, that in NT-3 infused axotomized animals, abundant p75 or NFM message is, with the exception of some neurons, colocalized in neurons displaying detectable trkC hybridization signal. Thus, as for NGF, multiple signals appear involved in regulation of neuronal phenotype by NT-3, including cell-specific factors in addition to the presence of the appropriate neurotrophin receptor. Further quantitative analysis of recent data demonstrating the ability of NT-3 to differentially regulate peptide and cjun mRNA levels (Gratto *et al.* 1994, 1995) will help confirm whether the restriction and specificity of NT-3's actions (at the concentrations used) is confined to that subpopulation of neurons expressing trkC.

4. MOLECULAR MECHANISMS UNDERLYING REGULATION OF PHENOTYPE BY NEUROTROPHINS IN INJURED DRG NEURONS

Reduction in the retrograde supply of peripherally derived neurotrophins is not the only signal responsible for alterations in neuronal function and phenotype observed after axotomy (Richardson & Lu 1994). In an apparently carefully orchestrated temporal and spatial pattern, alterations in intraganglionic, endoneurial and target expression of neurotrophins and cytokines occur. An adequate supply to the injured neurons of the correct trophic molecules to prevent cell death, regulate regeneration programmes and restore intact phenotype is probably necessary if functional recovery is expected. Chronic sciatic nerve transection results in a heterogenous, rapid and transient increase (2–4 days) in the concentrations of BDNF and interleukin-6 (IL-6) mRNA in neurons of the corresponding DRG (Karchewski *et al.* 1995*b*; Murphy *et al.* 1995), the former remaining slightly elevated in the case of crush injury (Ernfors *et al.* 1993; Sebert & Shooter 1993). There is also post-axotomy increased retrograde transport of ciliary neurtrotrophic factor (CNTF) and leukemia inhibitory factor (LIF) to the neurons, correlated with enhanced expression of LIF in proximal and distal segments of the crushed nerve and increased extracellular CNTF at the lesion site, despite a decrease in synthesis in the degenerating nerve (Friedman *et al.* 1992; Sendtner *et al.* 1992; Seniuk *et al.* 1992; Curtis *et al.* 1993, 1994). In addition to their potential role in survival, the axotomy-induced availability of the cytokine-related neurotrophic molecules and BDNF may serve to differentially modulate neuropeptide expression. Indeed, CNTF and LIF antagonize the effects of NGF on neuropeptide expression in sensory neurons *in vitro* (Mulderry 1994). It remains to be determined which other cellular programmes are altered.

In contrast to the injury-associated cytokines, whose actions and expression implicate them in regeneration, a clear role for neurotrophins in enhancing regeneration programmes is neither understood nor supported by experimental evidence (Diamond *et al.* 1987, 1992). Despite this, expression of NGF, BDNF and NT-4/5 are upregulated in peripheral nerve degenerating distal stump, and NT-3 mRNA levels remain detectable (Heumann *et al.* 1987; Meyer *et al.* 1992; Funakoshi *et al.* 1993). However, treatment of injured neurons with exogenous NGF or NT-3 (as described in this paper), alters the phenotype of responsive neurons so that they now more closely approximate the intact state, not the regenerating. After successful regrowth, regenerating axons presumably have access to neurotrophins which are produced in denervated target tissues and may assist in reestablishing normal phenotype (Funakoshi *et al.* 1993; Mearow *et al.* 1993).

Finally, recent evidence that systemic injection of NGF significantly increases DRG BDNF levels within 24 h (Apfel *et al.* 1994), prompted us to investigate the cellular source of this enhanced expression and determine whether regulation of one neurotrophin by another also occurs in injured neurons.

Figure 5 demonstrates that infusion of NGF for 1 week, 2 weeks after axotomy, results in dramatic upregulation in both the number and intensity of BDNF hybridization signal in intact neurons, with a more modest upregulation in injured neurons, the patterns of which coincide closely with trkA mRNA expression on the adjacent section. The consequences of upregulation in BDNF expression by NGF are not known, although it is likely to exert both paracrine and

nerve transection 3 weeks previously and NT-3 intrathecal infusion for the last week. Dashed lines divide the plots into quadrants where the upper right one contains data points representing neurons moderately to heavily labelled for both markers. In the normal DRG, approximately 32% and 30% of neurons fall into this category for trkC & p75 (*a*), or trkC & NFM (*e*) mRNA expression respectively; the number of heavily labelled neurons is markedly reduced by sciatic nerve transection for both sets of markers (*c*, *g*), but partially restored by NT-3 infusion (*d*, *h*). However, NT-3 infusion does not appear to counteract the downregulation of p75 or NFM mRNA in neurons that do not express detectable trkC mRNA and therefore, are presumably not NT-3 responsive. Upper right of each series (*b*, *f*)- photomicrographs of trkC (left) and p75 or NFM (right) *in situ* hybridization on adjacent sections of L5 DRG depicting neurons (arrows) ipsilateral to lesion which now display abundant trkC and p75 (*b*) or trkC and NFM (*f*) mRNA hybridization signal after NT-3 infusion. Magnification ×720.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

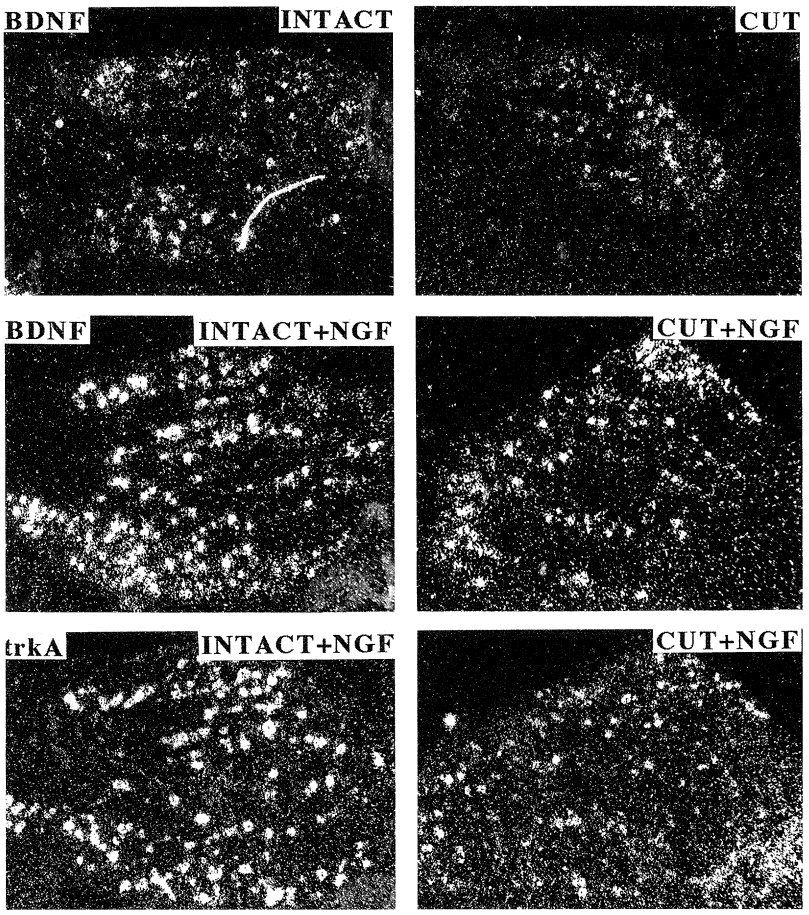


Figure 5. Upregulation of BDNF expression in intact and injured neurons by exogenous NGF. Dark-field photomicrographs of 5 μm L5 DRG sections (all from the same animal series) processed for *in situ* hybridization (Verge *et al.* 1995) to detect mRNAs as indicated at the top of each row. In intact DRG, BDNF mRNA expression is localized to a subpopulation of neurons of varying sizes. Three weeks after injury, the intensity and distribution of neurons expressing BDNF is not discernibly altered from that of the intact state. However, infusion of NGF for 1 week, 2 weeks after unilateral transection of the sciatic nerve results in a dramatic upregulation in both the number and intensity of hybridization signal detecting BDNF mRNA in intact neurons and a more modest upregulation in injured neurons, the patterns of which coincide closely with that of trkA mRNA expression on the adjacent section. Magnification ×30.

autocrine affects (see papers by A. L. Acheson & R. M. Lindsay, this volume); the former potentially on local interneurons in the sustantia gelatinosa which express full length trkB receptors (V. M. K. Verge, unpublished observations). Unlike NGF, NT-3 did not appear to influence BDNF expression (K. A. Gratto & V. M. K. Verge, unpublished observations), however, Rush *et al.* (1994) recently reported that exogenous BDNF enhanced levels of NT-3-like immunoreactivity in injured hypoglossal motor neurons. It is not known what other trophic factors are regulated by neurotrophins, but this phenomenon has important implications in understanding the complexity of downstream effects if these molecules are therapeutically applied.

5. CONCLUSIONS AND PERSPECTIVES

Results discussed here support roles for NGF and NT-3 in selectively maintaining or restoring many aspects of the differentiated phenotypes associated with the intact state in subpopulations of responsive adult DRG neurons after pathological insult. In this capacity, they hold promise for treatment of peripheral neuropathies (see papers by P. Anand and D. R. Tomlinson, this volume). However, more studies are required to fully understand the complexity of the regulation of mature phenotype by neurotrophins and their role in axonal growth, because an upset in the delicate balance of neurotrophic molecules necessary for the desired response can lead to undesireable consequences, such as the hyperalgesic response elicited by NGF (see papers by S. B. McMahon and C. Woolf this volume).

The authors thank Linda Andersen for expert technical help and Dr David Schreyer for critical review of the manuscript. This research was supported by grants from the MRC of Canada and the Saskatchewan Health Services Utilization and Research Commision. L.A.K. and K.A.G. are supported by University of Saskatchewan College of Medicine and Graduate Student Scholarships, respectively. V.M.K.V. is an MRC Scholar.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

REFERENCES

Aldskogius, H., Arvidsson, J. & Grant, G. 1992 Axotomy-induced changes in primary sensory neurons. In *Sensory neurons: diversity, development and plasticity* (ed. S. A. Scott), pp.363–383. New York: Oxford University Press.

Apfel, S. C., Dormia, C., Newell, M. E. & Kessler, J. A 1994 Nerve growth factor administration alters the expression of other neurotrophins in adult sensory ganglia. *Soc. Neurosci. Abstr.* **20**, 1302.

Chao, M. V. & Hempstead, B. L. 1995 p75 and Trk: a two-receptor system. *Trends Neusrosci.* **18**, 321–326.

Curtis, R., Adryan, K. M., Stark, J. L. *et al.* 1995 Differential role of the low affinity neurotrophin receptor (p75) in retrograde axonal transport of the neurotrophins. *Neuron* **14**, 1201–1211.

Curtis, R., Scherer, S. S., Somogyi, R. *et al.* 1994 Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. *Neuron* **12**, 191–204.

Curtis, R., Adryan, K. M., Zhu, Y., Harkness, P. J., Lindsay, R. M. & DiStefano, P. S. 1993 Retrograde axonal transport of ciliary neurotrophic factor is increased by peripheral nerve injury. *Nature, Lond.* **365**, 253–255.

Csillik, B., Schwab, M. E. & Thoenen, H. 1985 Transganglionic regulation of central terminals of dorsal root ganglion cells by nerve growth factor (NGF). *Brain Res.* **331**, 11–15.

Diamond, J., Foerster, A., Holmes, M. & Coughlin, M. 1992 Sensory nerves in adult rats regenerate and restore sensory function of the skin independently of endogenous NGF. *J. Neurosci.* **12**, 1467–1476.

Diamond, J., Coughlin, M., Macintyre, L., Holmes, M. & Visheau, B. 1987 Evidence that endogenous b nerve growth factor is responsible for the collateral sprouting, but not the regeneration, of nociceptive axons in adult rats. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6596–6600.

Ernfors, P., Rosario, C. M., Merlio, J.-P., Grant, G., Aldskogius, H. & Persson, H. 1993 Expression of mRNAs for neurotrophin receptors in the dorsal root ganglion and spinal cord during development and following peripheral or central axotomy. *Molec. Brain Res.* **17**, 217–226.

Fitzgerald, M., Wall, P. D., Goedert, M. & Emson, P. C. 1985 Nerve growth factor counteracts the neurophysiological and neurochemical effects of chronic sciatic nerve section. *Brain Res.* **332**, 131–141.

Fitzgerald, M., Woolf, C. J., Gibson, S. J. & Mallaburn, P. S. 1984 Alterations in the structure, function and chemistry of C fibres following local application of vinblastine to the sciatic nerve of the rat. *J. Neurosci.* **4**, 430–441.

Friedman, B., Scherer, S. S., Rudge, J. S. *et al.* 1992 Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells *in vivo*. *Neuron* **9**, 295–305.

Funakoshi, H., Frisen, J., Barbany, G., Timmusk, T., Zachrisson, O., Verge, V. M. K. & Persson, H. 1993 Differential expression of mRNAs for neurotrophins and their receptors after axotomy of the sciatic nerve. *J. cell Biol.* **123**, 455–465.

Goedert, M., Stoeckel, K. & Otten, U. 1981 Biological importance of the retrograde axonal transport of nerve growth factor sensory neurons. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5895–5898.

Gold, B. G., Storm-Dickerson, T. & Austin, D. R. 1993 Regulation of the transcription factor c-JUN by nerve growth factor in adult sensory neurons. *Neurosci. Letts.* **154**, 129–133.

Gratto, K. A., Friedman, B., Lui, L., Lindsay, R. M. & Verge, V. M. K. 1995 Further studies on the role of NT-3 in intact and injured primary sensory neurons. *Soc. Neurosci. Abstr.* **21**, 1056.

Gratto, K. A., Friedman, B., Lui, L., Lindsay, R. M. & Verge, V. M. K. 1994 Role of NT-3 in intact and injured sensory neurons. *Soc. Neurosci. Abstr.* **20**, 1098.

Heumann, R., Lindholm, D., Bandtlow, C. *et al.* 1987 Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages.*Proc. natn. Acad. Sci. U.S.A.* **84**, 8735–8739.

Hunt, S. P., Mantyh, P. W. & Priestley, J. V. 1992 The organization of biochemically qcharacterized sensory neurons. In *Sensory neurons: diversity, development, and plasticity* (ed. S. A. Scott), pp. 60–76. New York: Oxford University Press.

Inaishi, Y., Kashihara, Y., Sakaguchi, M., Nawa, H. & Kuno, M. 1992 Cooperative regulation of calcitonin gene-related peptide levels in rat sensory neurons via their central and peripheral processes. *J. Neurosci.* **12**, 518–524.

Ip, N., Stitt, T. N., Tapley, P. *et al.* 1993 Similarities and differences in the way neurotrophins interact with trk receptors in neuronal and nonneuronal cells. *Neuron* **10**, 137–149.

Kaplan, D. R., Martin-Zanca, D. & Parada, L. 1991 Tyrosine phosphorylation and tyrosine kinase activity of the trk protooncogene product induced by NGF. *Nature, Lond.* **350**, 158–160.

Karchewski, L. A., Hokfelt, T. & Verge V. M. K. 1995*a* Trilocalization, colocalization and plasticity of neurotrophin receptors in adult rat lumbar DRG neurons. (Submitted.)

Karchewski, L. A., Wetmore, C. & Verge V. M. K. 1995*b* Alterations in BDNF expression following injury. *Soc. Neurosci. Abstr.* **21**, 298.

Kashiba, H., Senba, E., Kawai, Y., Ueda, Y. & Tohyama M. 1992 Axonal blockade induces the expression of vasoactive intestinal polypeptide and galanin in rat dorsal root ganglion neurons . *Brain Res.* **577**, 19–28.

Klein, R., Jing, S., Nanduri, V., O'Rourke, E. & Barbacid, M. 1991 The trk protooncogene encodes a receptor for nerve growth factor. *Cell* **65**, 189–197.

Knyihar-Csillik, E., Kreutzberg, G. W., Raivich, G. & Csillik, B. 1991 Vasoactive intestinal polypeptide in dorsal root terminals of the rat spinal cord is regulated by the axoplasmic transport in the peripheral nerve. *Neurosci. Letts.* **131**, 83–87.

Korsching, S. & Thoenen, H. 1983 Nerve growth factor in sympathetic ganglia and corresponding target organs of the rat: correlation with density of sympatheticinnervation. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3513–3516.

Lamballe, F., Klein, R. & Barbacid, M. 1991 trkC, a new member of the trk family of tyrosine kinases, is a receptor for neurotrophin 3. *Cell* **66**, 976–979.

Lawson, S. 1992 Morphological and biochemical cell types of sensory neurons. In *Sensory neurons: diversity, development and plasticity* (ed. S. A. Scott), pp. 27–59. New York: Oxford University Press.

Lieberman, A. R. 1974 Some factors affecting retrograde neuroanl responses to axonal lesions. In *Essays on the nervous system* (ed. R. Bellairs & E. G. Gray), pp. 71–105. Oxford: Clarendon.

McMahon, S. B., Armanini, M. P., Lanway, H. L. & Phillips, H. S. 1994 Expression and coexpression of trk receptors in subpopulation of adult primary sensory neurons projecting to identified peripheral targets. *Neuron* **12**, 1161–1171.

Mearow, K. M., Kril, Y. & Diamond, J. 1993 Increased NGF mRNA expression in denervated rat skin. *Neuroreport* **4**, 351–354.

Mendell, L. M. 1995 Neurotrophic factors and the specification of neural function. *Neuroscientist* **1**, 26–34.

Meyer, M., Matsuoka, I., Wetmore, C., Olson, L. & Thoenen, H. 1992 Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J. cell Biol.* **119**, 45–54.

Mulderry, P. K. 1994 Neuropeptide expression by newborn and adult rat sensory neurons in culture: effects of nerve growth factor and other neurotrophic factors. *Neuroscience* **59**, 673–688.

Murphy, T. G., Grondin, J., Altares, M. & Richardson, P. M. 1995 Induction of interleukin-6 in axotomized sensory neurons. *J. Neurosci.* **15**, 5130–5138.

Raivich, G., Hellweg, R. & Kreutzberg, G. W. 1991 NGF receptor-mediated reduction in axonal NGF uptake and retrograde transport following sciatic nerve injury and during regeneration. *Neuron* **7**, 151–164.

Rich, K. M. Disch, S. P. & Eichler, M. E. 1989 The influence of regeneration and nerve growth factor on the neuronal cell body reaction to injury. *J. Neurocytol.* 18, 569–576.

Rich, K. M., Luszczynski, J. R., Osborne, P. A. & Johnson, E. M., Jr 1987 Nerve growth factor protects adult sensory neurons from cell death and atrophy caused by nerve injury. *J. Neurocytol.* **16**, 261–268.

Rich, K. M., Yip, H. K., Osborne, P. A., Schmidt, R. E. & Johnson, E. M., Jr 1984 Role of nerve growth factor in the adult dorsal root ganglia neuron and its response to injury. *J. Comp. Neurol.* **230**, 110–118.

Richardson, P. M. & Lu, X. 1994 Inflammation and axonal regeneration. *J. Neurol.* **241**, S57–S60.

Rush, R. A., Zhou, X.-F. & Greeson, D. 1994 Regulation of neurotrophin-3 by brain-derived neurotrophic factor in hypoglossal motor neurons. *Soc. Neurosci. Abstr.* **20**, 1482.

Sebert, M. E. & Shooter, E. M. 1993 Expression of mRNA for neurotrophic factors and their receptors in the rat dorsal root ganglion and sciatic nerve following nerve injury. *J. Neurosci. Res.* **36**, 357–367.

Sendtner, M., Stockli, K.A. & Thoenen, H. 1992 Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. *J. cell Biol.* **118**, 139–148.

Seniuk, N., Altares, M., Dunn, R. & Richardson, P. M. 1992 Decreased synthesis of ciliary neurotrophic factor in degenerating peripheral nerves. *Brain Res.* **572**, 300–302.

Snider, W. D. 1994 Functions of the neurotrophins during nervous system development: what the knockouts are teaching us. *Cell* **77**, 627–638.

Soppet, D., Escandon, E., Maragos, J. *et al.* 1991 The neurotrophic factors brain-derived neurotrophic factor and neurotrophin-3 are ligands for the trkB tyrosine kinase receptor. *Cell* **65**, 895–903.

Verge, V. M. K., Richardson, P. M., Wiesenfeld-Hallin, Z. & Hokfelt, T. 1995 Differential influence of nerve growth factor on neuropeptide expression *in vivo*: a novel role in peptide suppression in adult sensory neurons. *J. Neurosci.* **15**, 2081–2096.

Verge, V. M. K., Merlio, J.-P., Grondin, J. *et al.* 1992 Colocalization of NGF binding sites, trk mRNA, and low-affinity NGF receptor mRNA in primary sensory neurons: responses to injury and infusion of NGF. *J. Neurosci.* **12**, 4011–4022.

Verge, V. M. K., Tetzlaff, W., Bisby, M. A. & Richardson, P. M. 1990*a* Influence of nerve growth factor on neurofilament gene expression in mature primary sensory neurons. *J. Neurosci.* **10**, 2018–2025

Verge, V. M. K., Tetzlaff, W., Richardson, P. M. & Bisby, M. A. 1990*b* Correlation between GAP-43 and nerve growth factor receptors in rat sensory neurons. *J. Neurosci.* **10**, 926–934.

Verge, V. M. K., Richardson, P. M., Benoit, R. & Riopelle, R. J. 1989*a* Histochemical characterization of sensory neurons with high-affinity receptors for nerve growth factor. *J. Neurocytol.* **18**, 583–591.

Verge, V. M. K., Riopelle, R. J. & Richardson, P. M. 1989*b* Nerve growth factor receptors on normal and injured sensory neurons. *J. Neurosci.* **9**, 914–922.

Wetmore, C. & Olson, L. 1995 Neuronal and nonneuronal expression of neurotrophins and their receptors in sensory and sympathetic ganglia suggest new intercellular trophic interactions. *J. Comp. Neurol.* **353**, 143–159.

Wright, D. E. & Snider, W. D. 1995 Neurotrophin receptor mRNA expression defines distinct populations of neurons in rat dorsal root ganglia. *J. Comp. Neurol.* **351**, 329–338.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

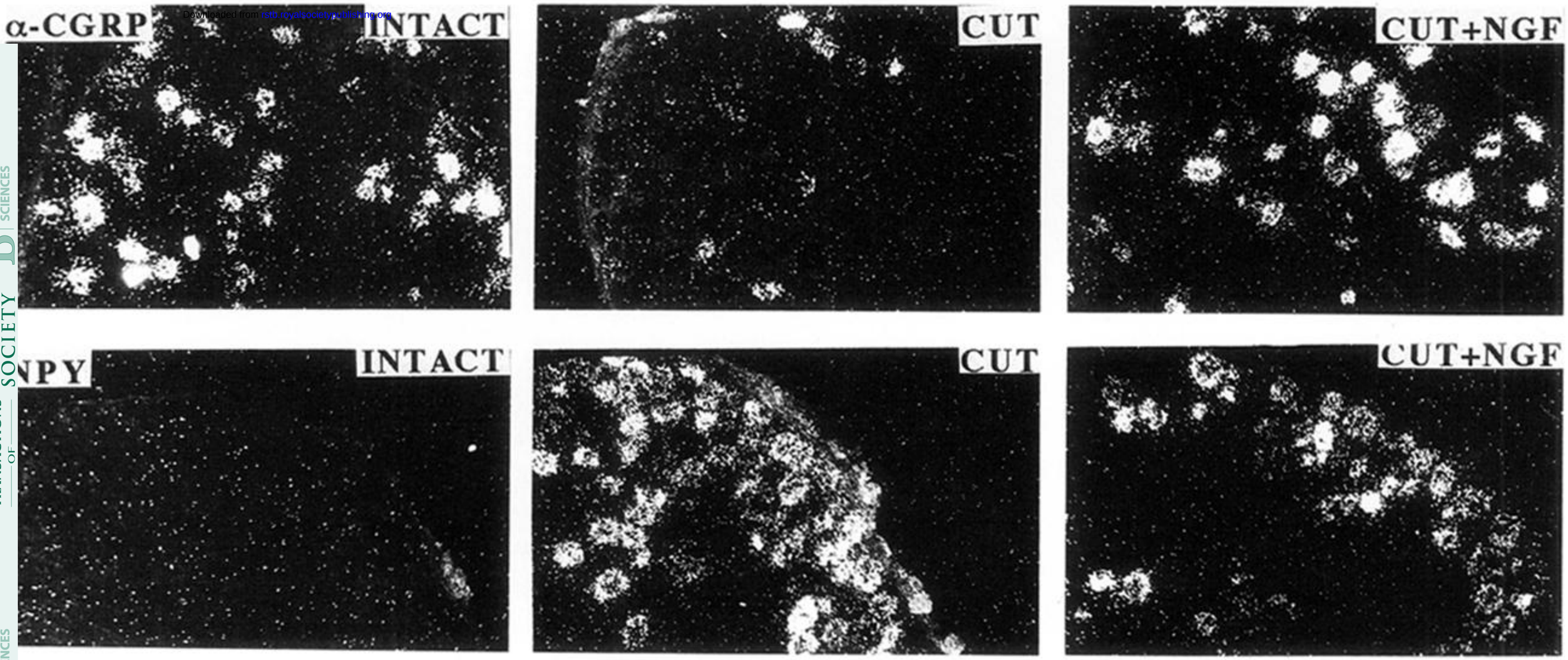


Figure 2. Differential influence of exogenous NGF on neuropeptide expression. Dark-field photomicrographs of L5 DRG sections (from the same animal series) processed for *in situ* hybridization using 35S-labelled oligonucleotide probes to detect α-CGRP (top row) and NPY (bottom row) mRNA and qualitatively demonstrate the ability of delayed intrathecal infusion of NGF (125–250 μg ml^{-1} h^{-1}) to injured neurons to both positively and negatively influence mRNA expression (Verge *et al.* 1995). In intact DRG many neurons express α-CGRP while few if any express NPY mRNA. Two weeks after injury, neuronal expression of α-CGRP is downregulated while NPY is heterogenously upregulated in injured neurons. Infusion of NGF for 7 days, 2 weeks after injury is effective in counteracting the axotomy-induced decrease in α-CGRP mRNA expression and mitigating the increase in NPY expression such that approximately one half fewer neurons now exhibit detectable hybridization signal for this peptide. Magnification ×57.

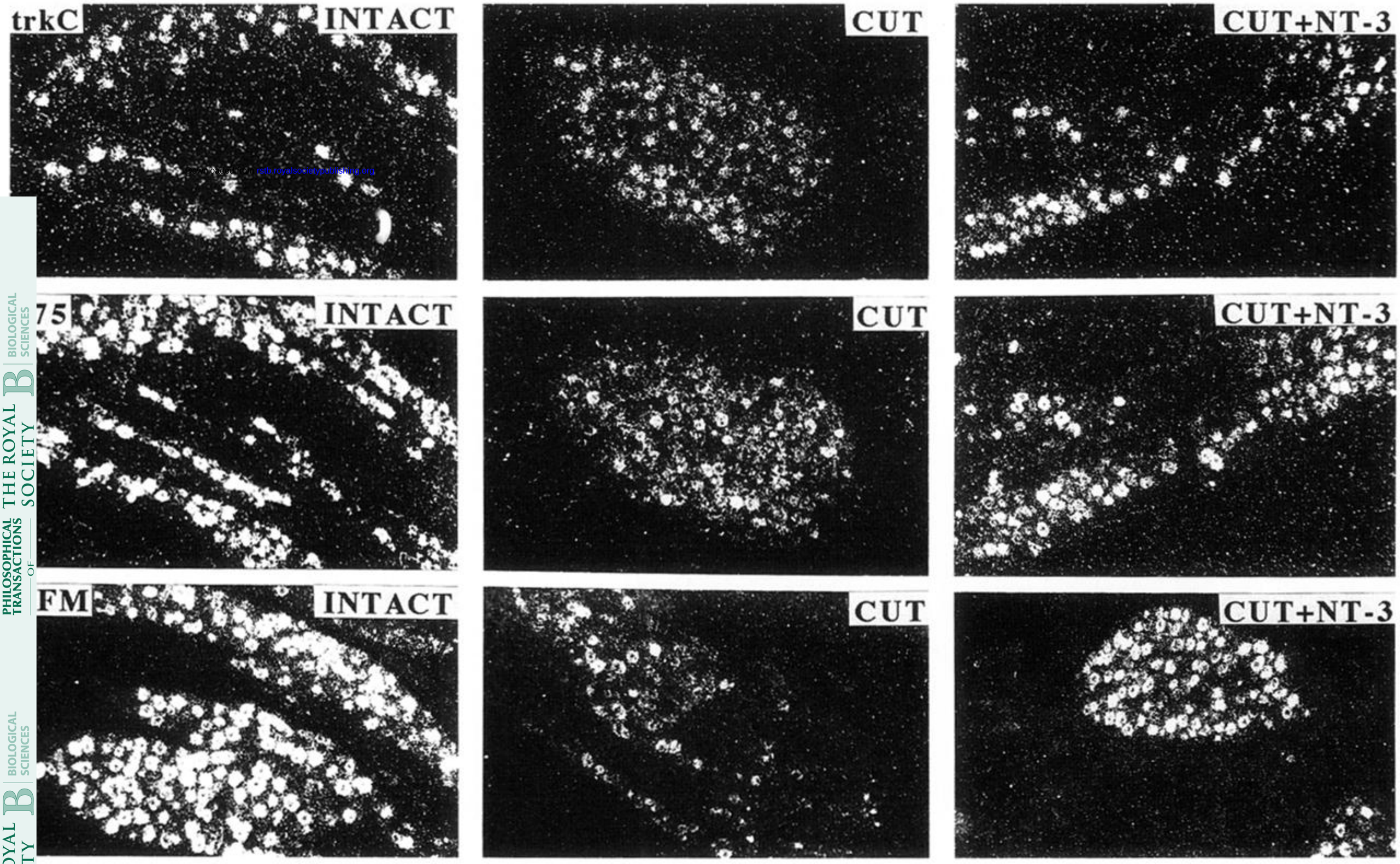
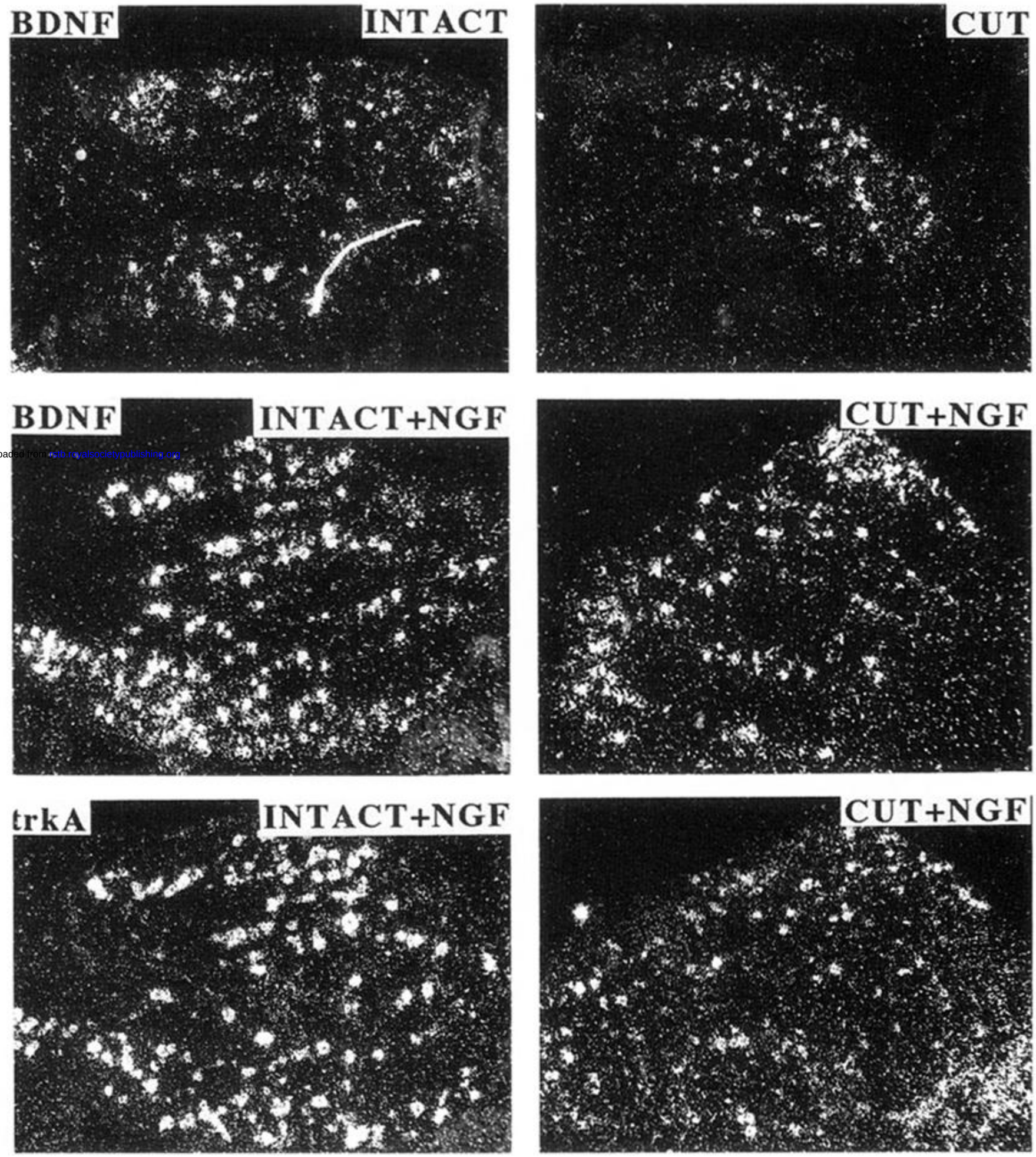


Figure 3. Ability of exogenous NT-3 to regulate NFM, p75 and trkC expression in injured lumbar sensory neurons (Gratto *et al.* 1994, 1995). Dark-field photomicrographs of 6 μm L5 DRG sections (all from the same animal series, p75 and trkC are adjacent sections) processed for *in situ* hybridization to detect mRNA as indicated in upper left of each row. In intact DRG NFM, p75 and trkC are abundantly and heterogenously expressed. Two weeks after injury, neuronal expression of all three messages is downregulated. Intrathecal infusion of NT-3 (600 ng μl^{-1} h^{-1}) for 7 days, 2 weeks after injury is effective in counteracting the axotomy-induced decrease in a subpopulation of the remaining injured neurons, quantitation of which is shown in figure 4. Magnification $\times 30$.

Figure 4. Quantification of data illustrated in figure 3. Scatterplots of labelling indices of 200–300 individual identified neurons in adjacent 6 μm thick sections of L5 DRG processed for either trkC (*x* axis, logarithmic scale), p75 (*y* axis, logarithmic scale) or NFM (*y* axis, logarithmic scale) *in situ* hybridization, to depict the relation between the two mRNAs examined in the upper (*a–d*) and lower series (*e–h*). Labelling index refers to the ratio of silver grain density over neuronal cytoplasm to grain density over areas of the neuropil devoid of positive hybridization signal. The left upper (*a*, *e*) and left lower (*c*, *g*) plots of each series pertain to left and right L5 DRG of a rat, with 3 week right sciatic nerve transection. The right lower plots of each series (*d*, *h*) describe the right L5 DRG of a rat with sciatic nerve transection 3 weeks previously and NT-3 intrathecal infusion for the last week. Dashed lines divide the plots into quadrants where the upper right one contains data points representing neurons moderately to heavily labelled for both markers. In the normal DRG, approximately 32 % and 30 % of neurons fall into this category for trkC & p75 (*a*), or trkC & NFM (*e*) mRNA expression respectively; the number of heavily labelled neurons is markedly reduced by sciatic nerve transection for both sets of markers (*c*, *g*), but partially restored by NT-3 infusion (*d*, *h*). However, NT-3 infusion does not appear to counteract the downregulation of p75 or NFM mRNA in neurons that do not express detectable trkC mRNA and therefore, are presumably not NT-3 responsive. Upper right of each series (*b*, *f*)- photomicrographs of trkC (left) and p75 or NFM (right) *in situ* hybridization on adjacent sections of L5 DRG depicting neurons (arrows) ipsilateral to lesion which now display abundant trkC and p75 (*b*) or trkC and NFM (*f*) mRNA hybridization signal after NT-3 infusion. Magnification ×720.



gure 5. Upregulation of BDNF expression in intact and injured neurons by exogenous NGF. Dark-field notomicrographs of 5 μm L5 DRG sections (all from the same animal series) processed for *in situ* hybridization erge *et al.* 1995) to detect mRNAs as indicated at the top of each row. In intact DRG, BDNF mRNA expression localized to a subpopulation of neurons of varying sizes. Three weeks after injury, the intensity and distribution of urons expressing BDNF is not discernibly altered from that of the intact state. However, infusion of NGF for 1 week, weeks after unilateral transection of the sciatic nerve results in a dramatic upregulation in both the number and tensity of hybridization signal detecting BDNF mRNA in intact neurons and a more modest upregulation in injured urons, the patterns of which coincide closely with that of trkA mRNA expression on the adjacent section. agnification ×30.