

Colocalization of NGF Binding Sites, *trk* mRNA, and Low-affinity NGF Receptor mRNA in Primary Sensory Neurons: Responses to Injury and Infusion of NGF

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The distributions of mRNAs for the protooncogene *trk* and the low-affinity NGF receptor (LNGFR) were studied by hybridization with oligonucleotide probes on sections of adult rat primary sensory and sympathetic ganglia. For comparison with high-affinity binding sites, adjacent sections were processed for NGF receptor radioautography. Among neurons in lumbar dorsal root ganglia and trigeminal ganglia, *trk* mRNA and NGF-binding sites were closely colocalized; this finding together with previous direct evidence in other cell types is taken to indicate that *trk* protein is an essential component of the high-affinity NGF receptor in adult sensory neurons. In lumbar dorsal root ganglia and trigeminal ganglia, abundant LNGFR mRNA was found in all neurons with strong ¹²⁵I-NGF labeling and on additional neurons lacking high-affinity NGF-binding sites. The presence of abundant LNGFR in neurons with high-affinity receptors could be the cause and/or consequence of their ability to respond to NGF. Neurons with abundant LNGFR mRNA but few high-affinity NGF-binding sites may have receptors for other members of the neurotrophin family. In nodose ganglia, neurons with high concentrations of LNGFR mRNA greatly outnumbered the small percentage with abundant *trk* mRNA. Following intrathecal infusion of NGF to otherwise normal dorsal root ganglia, the concentrations of LNGFR mRNA but not those of *trk* mRNA and NGF-binding sites were increased in NGF-responsive neurons. The usual single normal pattern of frequency histograms of LNGFR labeling indices became bimodal in response to NGF. Concentrations of NGF-binding sites, LNGFR mRNA, and *trk* mRNA were all decreased by peripheral nerve transection and restored by exogenous NGF, the restoration being complete for LNGFR mRNA and partial for *trk* mRNA and NGF-binding sites. The data indicate that NGF can regulate both LNGFR and *trk* mRNAs but do not clarify the possible contribution of the LNGFR protein to high-affinity binding sites.

The ability to bind NGF at high affinity and internalize NGF appears to be a stable property of a subpopulation of primary sensory neurons in the normal adult rat (Richardson and Riopelle, 1984; Verge et al., 1989a). The NGF-responsive population of lumbar dorsal root ganglion (DRG) neurons includes those with calcitonin gene-related peptide immunoreactivity and substance P immunoreactivity but not those with somatostatin immunoreactivity (Verge et al., 1989b). Little is known about genetic and developmental influences that determine which 40% of sensory neurons in the adult animal come to have functional high-affinity NGF receptors. However, the number of high-affinity NGF receptors on this fixed population of mature neurons has been shown to be reduced by axotomy and partially restored by infusion of exogenous NGF (Verge et al., 1989a). Prolonged administration of NGF can also increase the number of high-affinity NGF receptors on pheochromocytoma cells (Bernd and Greene, 1984).

Two molecules have been implicated in the high-affinity binding (Sutter et al., 1979), internalization, and signal transduction of NGF—the 140 kDa protein product of the protooncogene *trk* (Martin-Zanca et al., 1989) and the 75 kDa low-affinity NGF receptor (LNGFR) (Johnson et al., 1986; Radeke et al., 1987; Large et al., 1989). The *trk* protein binds NGF (Kaplan et al., 1991b), is quickly phosphorylated upon exposure to NGF (Kaplan et al., 1991a), and can mediate mitotic responses to NGF upon transfection into a quiescent fibroblast cell line lacking LNGFR (Cordon-Cardo et al., 1991). *trk* protein is generally agreed to be a necessary constituent of the functional NGF receptor (Loeb et al., 1991) in pheochromocytoma cells either as the entire receptor (Klein et al., 1991a; Weskamp and Reichardt, 1991) or as a major component of a receptor complex (Hempstead et al., 1991). LNGFR by itself mediates no biological action of NGF and is not necessary for several biological responses (Cordon-Cardo et al., 1991; Ibáñez et al., 1992). However, LNGFR may be required for high-affinity binding of NGF with equilibrium dissociation constant in the order of 10⁻¹¹ M (Hempstead et al., 1991) and is present in all neurons known to respond physiologically to NGF by survival and neurite extension. In short, *trk* protein is an important component of the functional NGF receptor and the contribution of LNGFR is still somewhat ambiguous.

At least some neurons in most sensory ganglia respond to NGF. The same statement is true for BDNF (brain-derived

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neurotrophic factor), NT-3 (neurotrophin-3), or NT-5, although the proportions responding to the four neurotrophins vary among ganglia (Davies et al., 1986; Ernfors et al., 1990; Berkemeier et al., 1991). LNGFR binds BDNF and NT-3 as well as NGF with low affinity and has been proposed to be a common component of diverse high-affinity receptors for NGF-like molecules (Ernfors et al., 1990; Rodriguez-Tébar et al., 1990). *trk* protein is not a receptor for BDNF but shows some responses to NT-3 and NT-5 (Berkemeier et al., 1991; Cordon-Cardo et al., 1991). Other members of the *trk* family, *trk B* and *trk C*, appear to be responsible for signal transduction of BDNF and NT-3 (Klein et al., 1991b; Lamballe et al., 1991; Soppet et al., 1991; Squinto et al., 1991).

In an attempt to clarify the roles of LNGFR and *trk* in high-affinity NGF binding, the distributions of NGF-binding sites, LNGFR mRNA, and *trk* mRNA were correlated for rat primary sensory neurons in several ganglia. Also, the effects of sciatic nerve transection and intrathecal infusion of NGF were analyzed in lumbar DRG neurons and correlated with earlier observations with NGF receptor radioautography (Verge et al., 1989a).

Materials and Methods

Surgery and tissue preparation. Experiments were performed on Sprague-Dawley rats of either sex weighing approximately 200 gm and anesthetized for surgery with pentobarbital, 50 mg/kg intraperitoneally. In 28 rats, the right sciatic nerve was transected at its origin from the L4 and L5 spinal nerves and one-half of these rats were infused immediately or subsequently with β -NGF, prepared from mouse submandibular glands by cation-exchange chromatography (Longo et al., 1989). For administration of NGF, an osmotic pump (Alza, 2001) was inserted in the dorsal lumbar subcutaneous space and attached to silicon tubing (0.3 mm o.d.) inserted 3 cm into the subarachnoid space from the lumbosacral junction. NGF was delivered at 125 ng/hr in a solution of phosphate-buffered saline containing bovine serum albumin (1 mg/ml), streptomycin (100 U/ml), and penicillin (100 U/ml). Control rats were infused with buffered solution alone. Three to four weeks after sciatic nerve transection and 1 week after insertion of osmotic pumps, rats were deeply anesthetized and perfused per aorta with phosphate-buffered saline. The right and left L5 DRG (fifth lumbar dorsal root ganglia) were frozen at -60°C in one mold containing Tissue-Tek (Miles Laboratories). Before sectioning, blocks containing DRG from NGF-infused and uninfused rats were fused by embedding medium so that four DRG could be sectioned on the same chuck and mounted on the same slide. In addition to lumbar DRG, nodose, trigeminal, and superior cervical ganglia were removed from normal rats and frozen in the same molds. Sections from DRG, nodose, trigeminal, and sympathetic ganglia were cut on a cryostat set at $5\ \mu\text{m}$ and mounted on gelatin-coated slides or commercially coated slides (Probe-on, Fisher). Sections were stored at -80°C until use.

NGF receptor radioautography. As described in previous publications (Richardson et al., 1989; Verge et al., 1989a), sections were incubated for 90 min at room temperature in a solution of 30–80 pM ^{125}I -NGF in phosphate-buffered 0.1 M saline containing magnesium chloride (0.5 mM), cytochrome C (1 gm/liter), phenylmethylsulfonyl fluoride (0.5 mM), and leupeptin (4 mg/liter). Slides were rinsed in several changes of cold buffer over 3 min, fixed in buffered 2% formaldehyde/2% glutaraldehyde, defatted in alcohols and xylene, rehydrated, and dried. They were then dipped in Kodak NTB-2 emulsion diluted with equal volume of distilled water, exposed in the dark for 4–10 d, developed, stained with toluidine blue, and mounted with a coverslip.

In situ hybridization. *In situ* hybridization with oligonucleotide probes for LNGFR and *trk* was performed on sections adjacent to those used for NGF receptor radioautography.

A 50-mer DNA oligonucleotide, 5'-ACAAGGCCACGACCACAGCAGCCAAGATGGAGCAATAGACAGGAATGAG-3', was prepared complementary to the cDNA encoding the highly conserved transmembrane segment of LNGFR (Ernfors et al., 1989) and labeled with ^{35}S -dATP by the terminal transferase method (Ausubel et al., 1987). Cryostat sections taken from the freezer were fixed for 30 min in 4% paraformaldehyde, rinsed in buffered saline, dehydrated in alcohols,

deproteinized with chloroform for 3 min, rinsed in alcohol, and dried in air. The sections were hybridized overnight at 42°C in 1,000,000 cpm of probe in 100 μl of a solution containing formamide (50%), $4\times$ saline-sodium citrate (SSC), dextran sulfate (100 gm/liter), sarcosyl (1%), salmon sperm DNA (250 mg/liter), yeast tRNA (500 mg/liter), dithiothreitol (DTT; 50 mM), and phosphate buffer (0.02 M, pH 7.0). They were then washed four times with $1\times$ SSC for 15 min at 55°C , rinsed very briefly in distilled water, dried, dipped in Kodak NTB2 emulsion diluted 1:1 with distilled water, exposed in the dark at 4°C for 7–14 d, developed, stained with toluidine blue, and mounted with a coverslip.

For *trk* hybridization, an antisense oligonucleotide to rat *trk* mRNA 5'-AAGGTTGAACTCAAAGGGTTGTCCATGAAGGCAGCCATGATGGAGGC-3' (J.-P. Merlio, P. Ernfors, and H. Persson, unpublished observations; Meakin et al., 1992) was labeled at the 3' end with ^{35}S -dATP. The rat *trk* oligonucleotide is the counterpart of base pairs 1198–1245 in the human *trk* sequence (Martin-Zanca et al., 1989) lying between the second immunoglobulin domain (Schneider and Schweiger, 1991) and the transmembrane domain. In this region of the rat *trk* protein, 9 of 17 amino acids are homologous with those in *trk B* and 4 of 17 are homologous with those in *trk C*. As previously described (Dagerlind et al., 1990), sections were removed from the freezer, dried for 20–40 min at room temperature, and hybridized overnight at 42°C with 10^7 cpm of ^{35}S -labeled probe in 1 ml of hybridization solution containing formamide (50%), $4\times$ SSC, dextran sulfate (100 gm/liter), sarcosyl (1%), 0.02 M phosphate buffer, salmon sperm DNA (500 gm/liter), and DTT (200 mM). Following hybridization, slides were washed four times with $1\times$ SSC for 15 min at 55°C , brought to room temperature in the final rinse, rinsed briefly in distilled water, and quickly dehydrated in ascending concentrations of alcohol. Tissue sections were dipped in NTB2 emulsion diluted 1:1 in distilled water, exposed in the dark for 6–10 d, developed, stained with toluidine blue, and mounted with coverslips.

Quantification. Radioautographic labeling was quantified on sections processed for NGF receptor radioautography or *in situ* hybridization with *trk* or LNGFR probes. Under oil immersion light microscopy with an interactive image analysis system (Richardson et al., 1989), the percentages of cytoplasmic area covered by silver grains were measured for individual numbered neurons in both preparations of each ganglion. For each image, the density threshold was adjusted interactively so that the area per grain was constant for all neurons. Correction for grain overlap was made to obtain a parameter linearly related to density of silver grains (Richardson et al., 1989). The term labeling index refers to the ratio of grain density over neuronal cytoplasm to grain density over areas of the ganglia without neuronal cell bodies. Software for the image analysis system was generously provided by W. G. Tatton (University of Toronto) and supplemented with published programs for data analysis (Press et al., 1988). In colocalization studies of lumbar DRG, nodose ganglia, or trigeminal ganglia, montages of photomicrographs (magnification, $400\times$) were prepared from pairs of adjacent sections, one processed for receptor radioautography and the other for hybridization with *trk* or LNGFR probes. Individual neurons with visible nucleolus in one of the two sections were identified, numbered, and quantified in each of the two sections. To avoid slide-to-slide variability, comparisons to determine the effect of nerve transection and/or NGF infusion were made only on sections mounted on the same slide.

Results

Normal DRG

As previously reported, high-affinity binding sites were heterogeneously distributed among neurons in normal L5 DRG (Fig. 1). Frequency histograms of labeling densities in NGF receptor radioautographs have been interpreted to indicate the presence of two populations of neurons, approximately 40% with and 60% without high-affinity receptors (Verge et al., 1989a).

In situ hybridization with the LNGFR probe yielded labeling that varied considerably among neurons. The distributions of the logarithms of LNGFR labeling indices in normal DRG seemed consistently to fit single normal distributions (Fig. 2A) with no suggestion of two populations. In colocalization studies of two normal L5 DRG (Fig. 2C,E), neurons with ^{125}I -NGF binding labeling indices of >3 had LNGFR hybridization la-

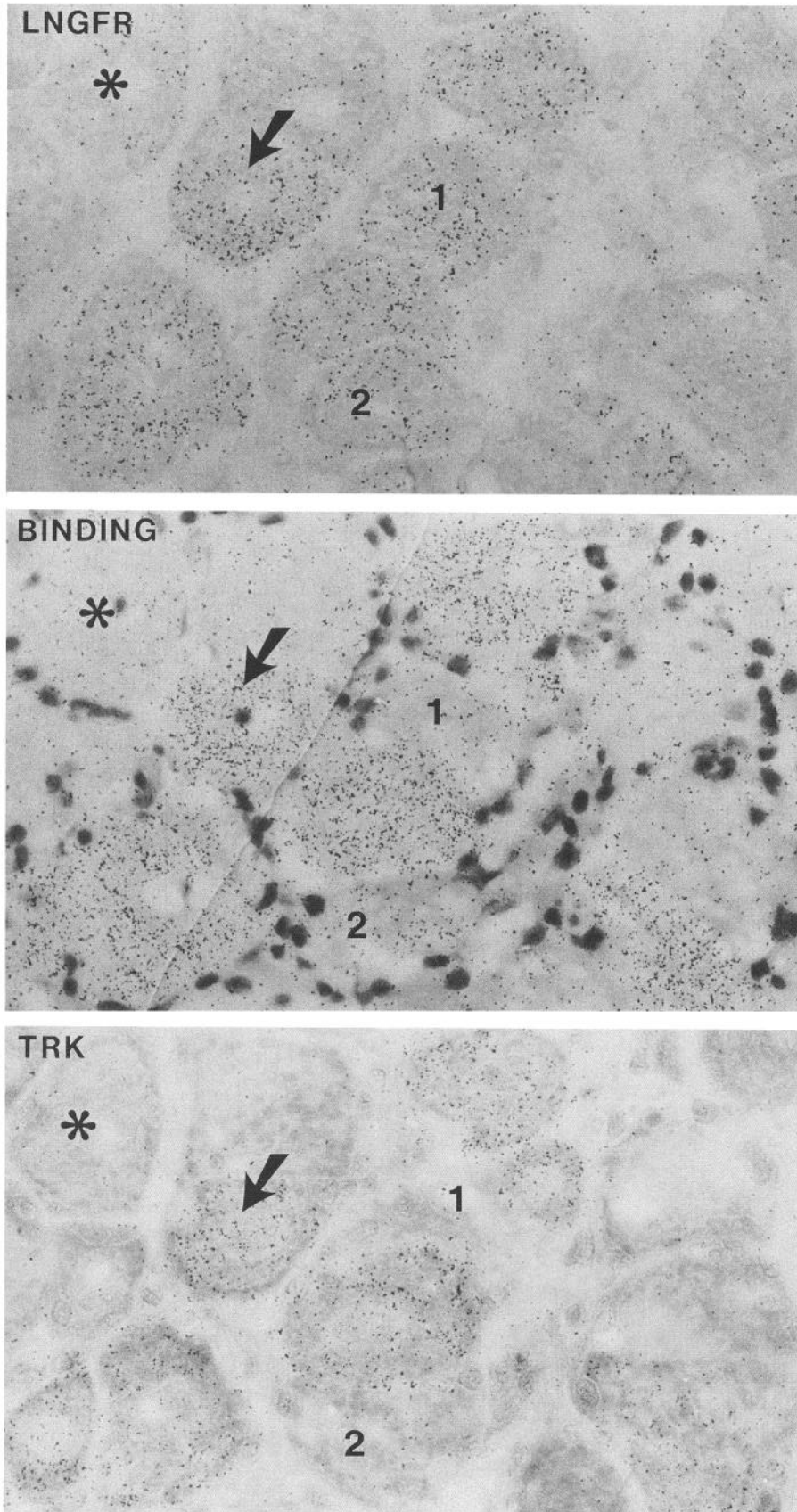


Figure 1. Photomicrographs showing adjacent cryostat sections, 5 μ m thick, of an L5 DRG processed for LNGFR hybridization (*top*), NGF receptor radioautography (*middle*), and trk hybridization (*bottom*). The neurons are heterogeneously labeled in all three preparations. Note the close correlation between strong expression of trk and the presence of high-affinity NGF-binding sites. All neurons with abundant trk mRNA also have abundant LNGFR mRNA and many ¹²⁵I-NGF binding sites (*arrows*). All neurons with little or no LNGFR mRNA (*asterisks*) and a few neurons with abundant LNGFR mRNA (*1* and *2*) have undetectable concentrations of trk mRNA and high-affinity NGF-binding sites. Magnification, 610 \times .

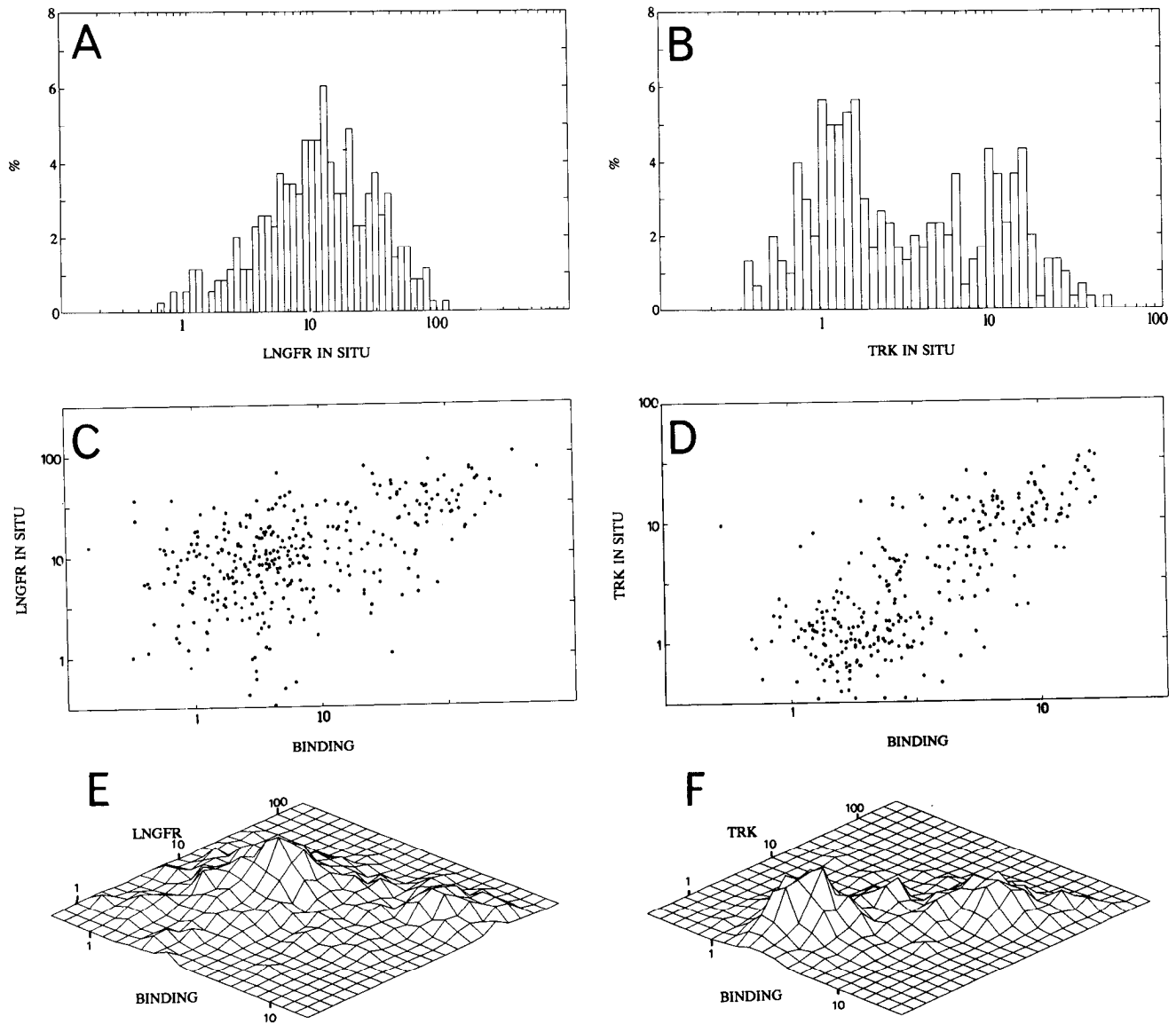


Figure 2. Frequency histograms of labeling indices (*A, B*), scatter plots (*C, D*), and three-dimensional histograms (*E, F*, with neuronal frequency as the z-axis) to quantify labeling of identified neurons in adjacent sections of L5 DRG processed for NGF receptor radioautography and either LNGFR hybridization (*A, C, E*) or trk hybridization (*B, D, F*). The frequency histogram of LNGFR labeling indices is unimodal (*A*). Strongly NGF-positive neurons invariably are strongly LNGFR positive, but some neurons have abundant LNGFR mRNA and few NGF binding sites (*C, E*). The frequency histogram of trk labeling is bimodal (*B*). Labeling indices with trk hybridization and receptor radioautography are highly correlated, and two populations can be discerned with or without abundant trk mRNA and high-affinity NGF receptors (*D, F*).

being indices virtually always >3 and usually >10 . The converse was not true as there were many neurons with LNGFR labeling indices of >10 but near background labeling in NGF receptor radioautography.

With trk hybridization, labeling of L5 DRG neurons was again heterogeneous, ranging from below background to $>100\times$ background. Frequency histograms of the logarithms of trk labeling indices prepared for more than 200 neurons in each of three L5 DRG showed bimodal distributions (Fig. 2*B*). In colocalization analysis of one DRG (Fig. 2*D, F*), most neurons were situated within two clusters with mean ^{125}I -NGF labeling indices of 2 and 8 and mean trk labeling indices of 1 and 10, respectively. Upon curve fitting of trk frequency histograms to a double normal distribution with the Levenberg-Marquardt algorithm (Press et al., 1988), 60% (54–67%) were lightly labeled with mean index 1.5 (1.1–2.2) and 40% were heavily labeled with mean index 25

(9.2–54). All three DRG contained some intermediately labeled neurons that could not confidently be classified into one or the other groups.

Trigeminal, nodose, and sympathetic ganglia

For both NGF-receptor radioautography and LNGFR hybridization, results for trigeminal ganglia were similar to those for L5 DRG (Figs. 3, 4). Again, those neurons with NGF labeling indices of >3 almost always had LNGFR labeling indices of >3 but a population of neurons with abundant LNGFR hybridization and little binding could be clearly detected (Fig. 5*E*). The tight colocalization of trk mRNA and NGF-binding sites that was documented for lumbar DRG also appeared to be valid for trigeminal neurons (Fig. 4), although these data were not rigorously quantified.

A marked discrepancy between NGF binding or trk hybrid-

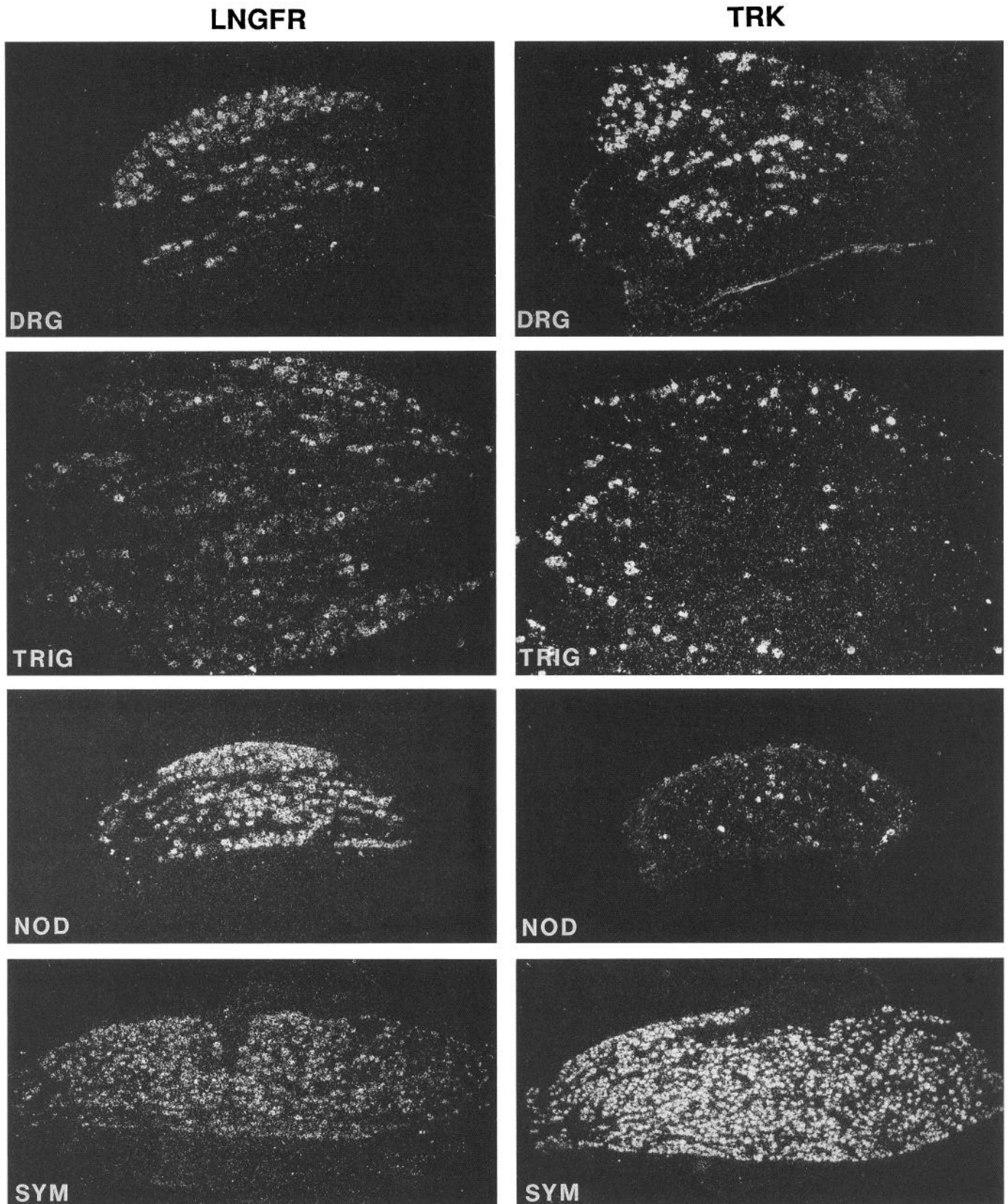


Figure 3. Dark-field photomicrographs of sections of lumbar DRG, trigeminal ganglia (*TRIG*), nodose ganglia (*NOD*), and sympathetic ganglia (*SYM*), all cut on the same chunk to show neuronal distributions and relative concentrations, are illustrated for LNGFR mRNA (*left*) or *trk* mRNA (*right*). For all three sensory ganglia, the expression of both LNGFR and *trk* is heterogeneous among neurons. In nodose ganglia, *trk* is strongly expressed in fewer neurons than in lumbar DRG or trigeminal ganglia and LNGFR is expressed at least as strongly in at least as many neurons. Virtually all sympathetic neurons are heavily labeled in both hybridizations. Magnification, 40 \times .

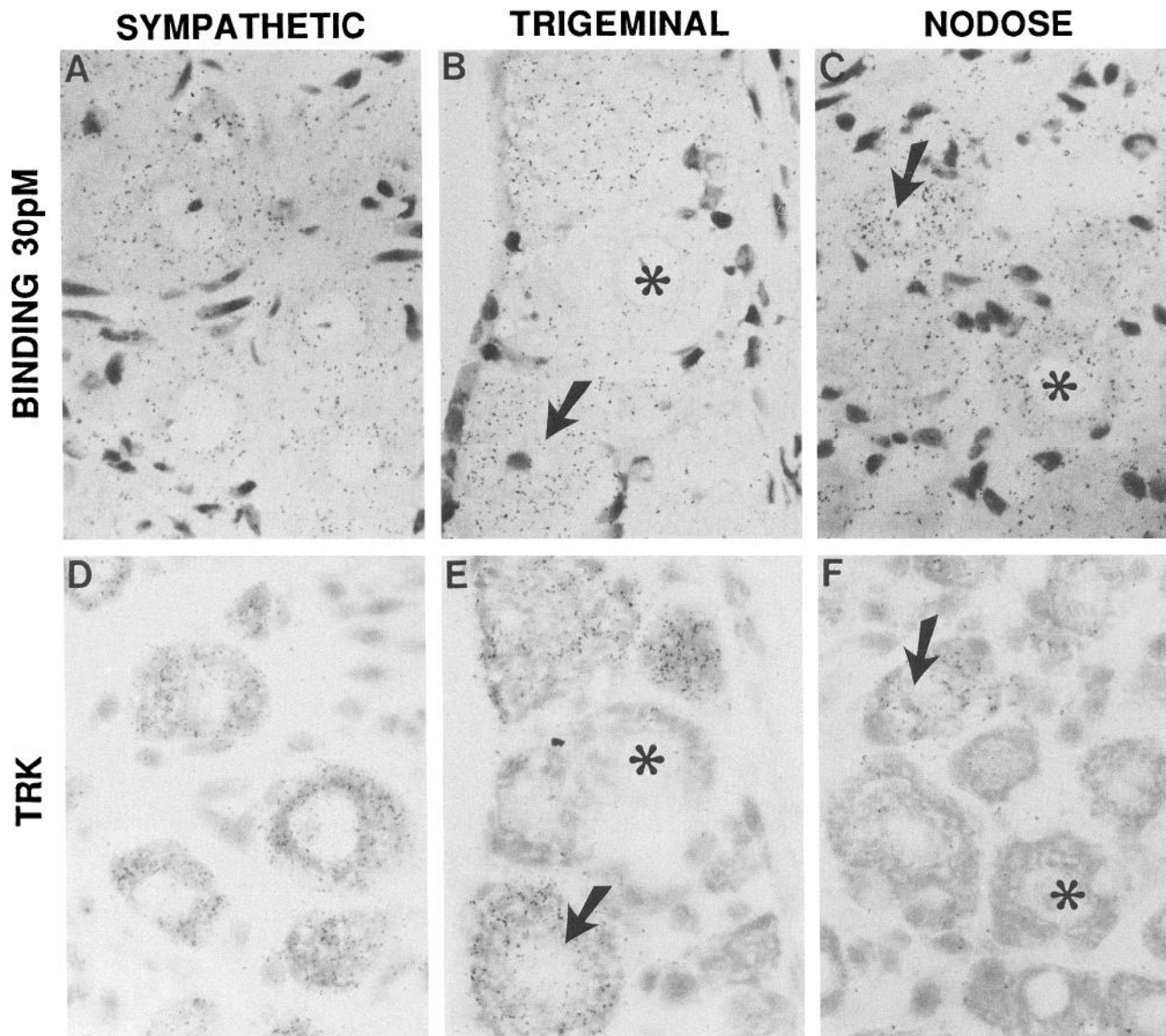


Figure 4. Photomicrographs of adjacent sections, 5 μ m thick, of sympathetic (*A, D*), trigeminal (*B, E*), and nodose (*C, F*) neurons on a pair of slides, one processed for NGF receptor radioautography (*A–C*) and the other for *in situ* *trk* hybridization (*D–F*). Most or all sympathetic neurons have both high-affinity NGF-binding sites and strong expression of *trk*. Individual trigeminal neurons show comparable labeling with each procedure with either strong (arrows) or near-background labeling (asterisks). In the nodose ganglion, neurons with strong expression of *trk* are heavily labeled with 125 I-NGF (arrows) but some neurons with little or no *trk* mRNA (asterisks) have more 125 I-NGF labeling than corresponding cells in the trigeminal ganglion. Magnification, 700 \times .

ization and LNGFR hybridization was apparent in nodose neurons (Fig. 3). The percentage of neurons with strong expression of *trk* was estimated to be 5% in nodose ganglia, considerably less than in DRG or trigeminal ganglia (Fig. 4). In contrast, the frequency histograms of the logarithms of LNGFR labeling indices in two nodose ganglia were rather similar to those for DRG and trigeminal ganglia, fitting single normal distributions (Fig. 5*D*). The mean density of LNGFR labeling appeared to be as high or higher in nodose ganglia than in DRG when sections from the two ganglia were processed on the same slide (Fig. 3). More than one-half of the neurons in nodose ganglia had abundant LNGFR mRNA but little or no *trk* mRNA (Fig. 3). In NGF receptor radioautography preparations of nodose ganglia (Fig. 5*B*), the percentage of neurons with dense labeling, presumably with high-affinity receptors, was difficult to define

precisely because of overlap with a larger population of neurons with intermediate labeling.

Virtually all neurons in sympathetic ganglia displayed abundant NGF-binding sites, LNGFR mRNA, and *trk* mRNA (Figs. 3, 4).

Nerve injury and NGF infusion

The changes of NGF binding sites in sensory neurons following axotomy and/or infusion of NGF (Figs. 6, 7) were consistent with those described in a previous publication (Verge et al., 1989a). Two to three weeks after sciatic nerve transection, the density of high-affinity NGF-binding sites on L5 DRG neurons was decreased and the normal bimodal pattern became unimodal with loss of the more heavily labeled population (Fig. 7*D*). NGF infusion partially reversed the effects of axotomy on

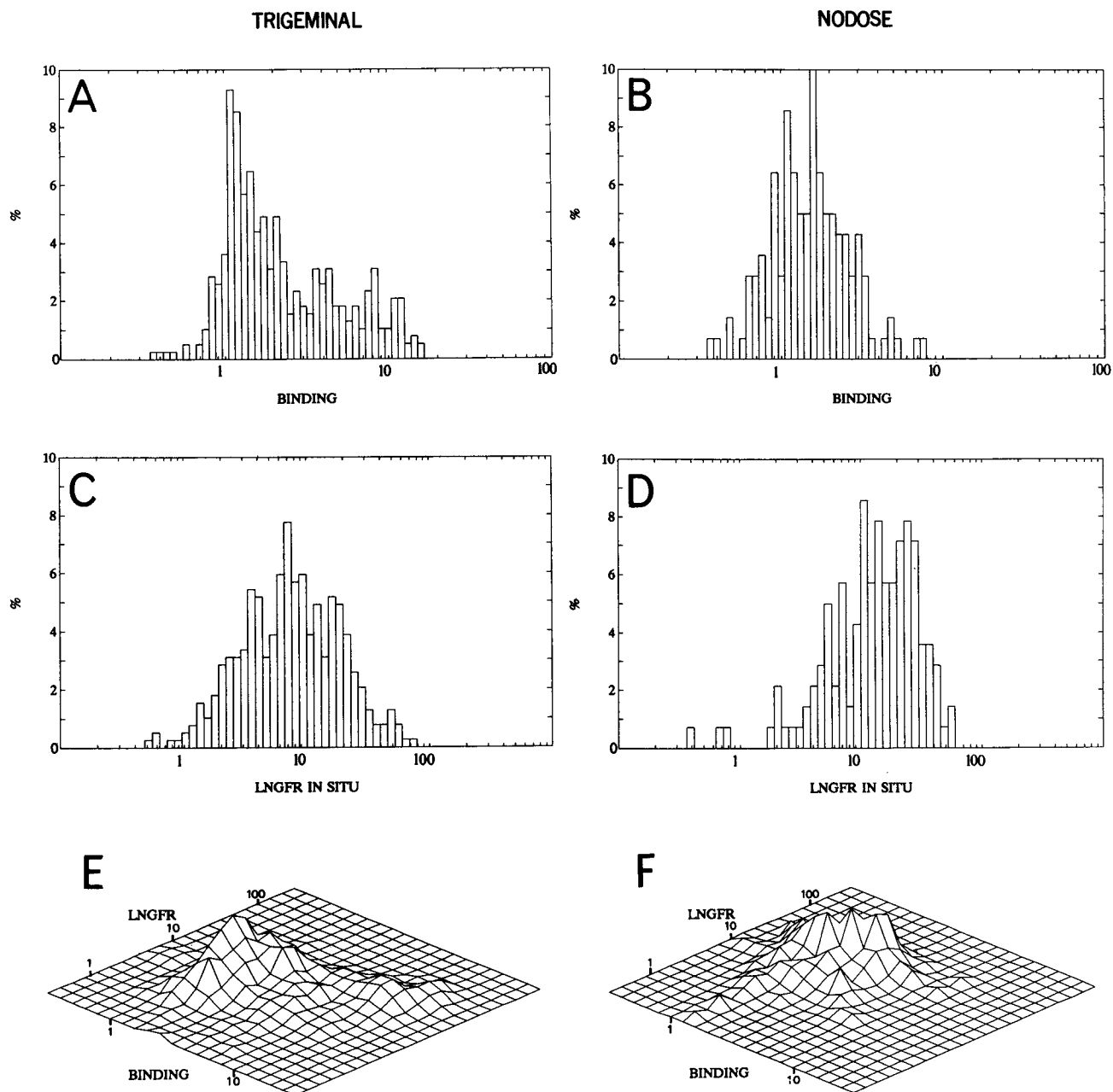


Figure 5. Frequency histograms of neuronal labeling by LNGFR hybridization (*C, D*) and NGF receptor radioautography (*A, B*) plus three-dimensional histograms (*E, F*) to show the relationship between the NGF binding sites and LNGFR mRNA in a trigeminal and nodose ganglion. In the trigeminal ganglion, the two markers are moderately well correlated although not all neurons with strong LNGFR labeling have high-affinity NGF receptors (*E*). In the nodose ganglia, LNGFR labeling appears to be more prominent than in the trigeminal ganglion but neurons with high-affinity binding sites are scarce (*F*).

binding sites (Fig. 7*K*) but did not appreciably influence NGF binding to neurons with intact axons (Fig. 7*H*). For the most heavily labeled one-third of neurons, which normally bear high-affinity receptors, the mean labeling index fell to 50% of normal after axotomy and returned to 76% with infusion of NGF. As calculated previously (Verge et al., 1989a), the changes in total numbers of receptors are considerably greater than suggested by these labeling indices.

Three to four weeks following sciatic nerve transection, labeling with the LNGFR probe was consistently diminished in all ipsilateral L5 DRG examined (Fig. 7*D*). This diminution was evident by 2 d and persisted at least 9 weeks after injury (data not shown). NGF infusion restored LNGFR labeling to slightly

above normal values in axotomized neurons (Fig. 7*J*) and resulted in supranormal LNGFR labeling of otherwise normal DRG (Fig. 7*G*). Two series of LNGFR hybridizations were quantified with mean labeling indices of 54%, 200%, and 112% of normal after nerve transection, NGF infusion without nerve transection, and nerve transection plus NGF infusion, respectively. The frequency histograms of the logarithms of LNGFR labeling indices became bimodal after NGF infusion (Fig. 7*G*). In colocalization analyses, NGF infusion was shown to increase LNGFR mRNA in the one-third of neurons most densely labeled with ^{125}I -NGF but not in the least heavily labeled third (Fig. 8).

trk labeling of L5 DRG neurons was consistently reduced by

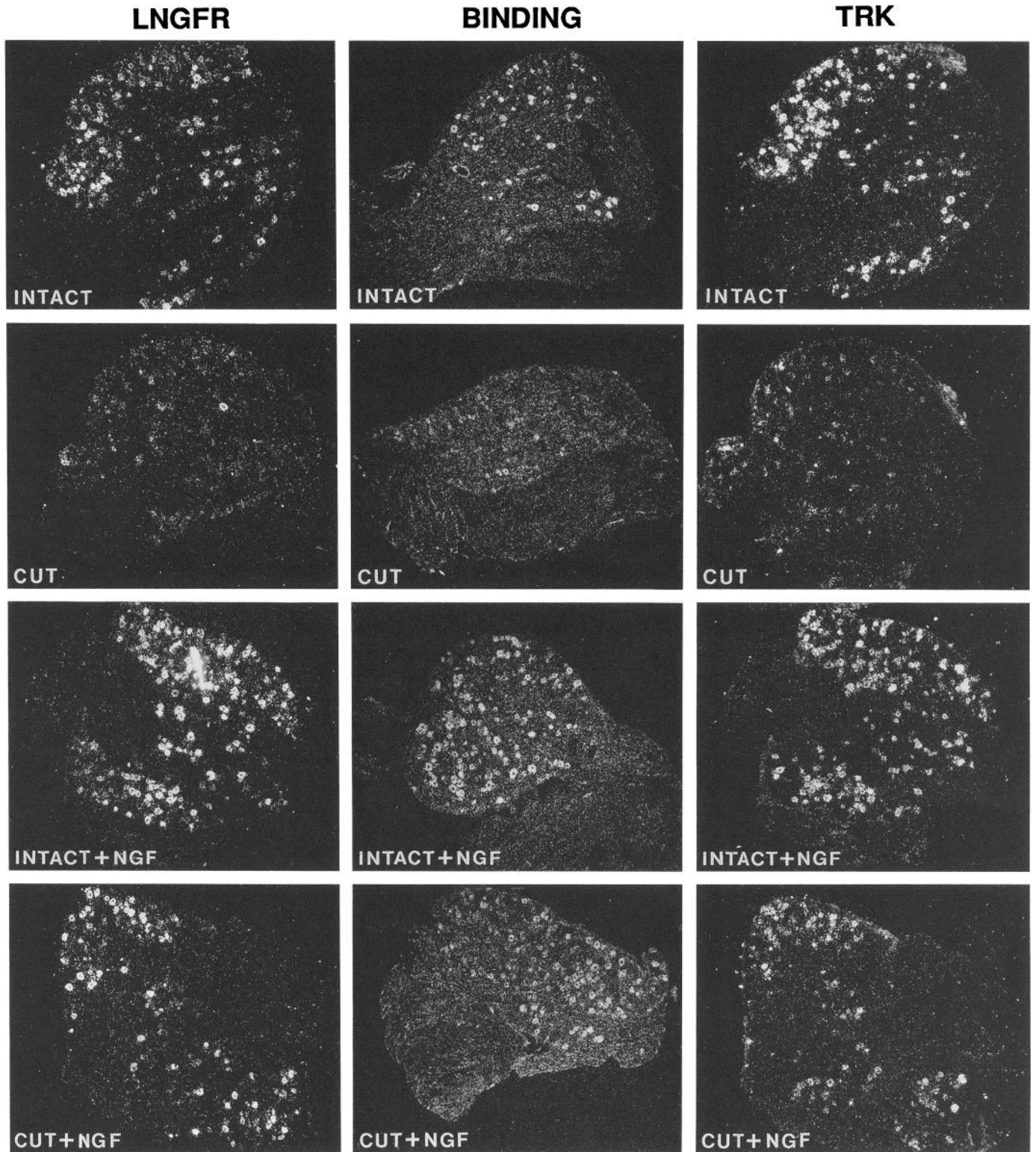


Figure 6. Dark-field photomicrographs to show sections of L5 DRG processed for LNGFR hybridization or *trk* hybridization and L4 DRG from the same animals processed for NGF receptor radioautography (*BINDING*). The DRG are associated with normal sciatic nerves or sciatic nerves cut 3 weeks previously and are exposed to intrathecally infused NGF or control solution infused from 14–21 d after axotomy. Nerve transection decreases the labeling in all three preparations and its effects are, to greater or lesser extent, counteracted by exogenous NGF. NGF infusion superinduces LNGFR mRNA in otherwise normal ganglia but has little or no effect on NGF binding sites or *trk* mRNA. Magnification, 35 \times .

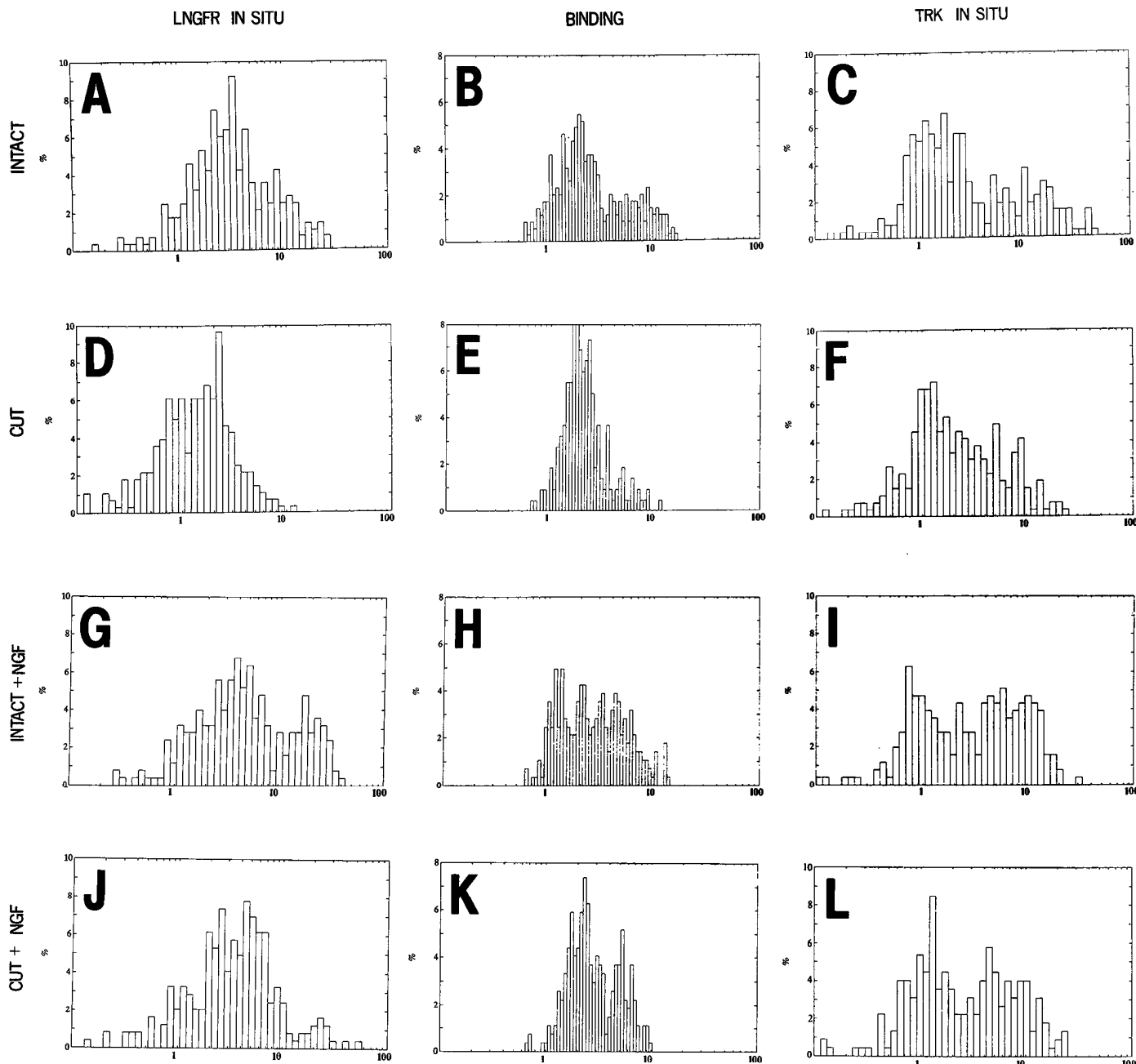


Figure 7. Quantification of the type of data illustrated in Figure 6. Frequency histograms of LNGFR, NGF (*BINDING*), and *trk* labeling indices are plotted for pairs of ganglia associated with nerve transection 3 weeks previously and/or NGF infusion from 14–21 d after axotomy. Data for LNGFR hybridization and NGF-receptor radioautography are from adjacent sections; *trk* hybridization is from another series of ganglia. The frequency histogram of LNGFR binding is unimodal for a normal ganglion (*A*) and becomes bimodal after NGF infusion (*G*). The frequency histograms of NGF receptor radioautography and *trk* hybridization are bimodal for normal ganglia (*B*, *C*) and become unimodal after nerve transection (*E*, *F*).

nerve transection (Fig. 7*F*) and partially restored by NGF infusion (Fig. 7*L*). Administration of NGF did not increase *trk* labeling above normal values in uninjured neurons (Fig. 7*I*). In quantification of two series, *trk* labeling for the most heavily labeled one-third of neurons was 48% of normal after nerve transection and 61% of normal after transection plus NGF infusion. Frequency histograms of *trk* labeling resembled those of NGF labeling indices, with the bimodal patterns of normal ganglia becoming unimodal after nerve transection (Fig. 7*F*) and bimodal again after NGF infusion (Fig. 7*L*).

Discussion

The distribution of LNGFR mRNA among normal rat primary sensory neurons is more widespread than that of high-affinity NGF-binding sites. We speculate that those sensory neurons that have high concentrations of LNGFR mRNA but lack functional NGF receptors have receptors for other neurotrophins. Such neurons are particularly abundant in the nodose ganglia where both NT-3 and BDNF promote the survival of significant percentages of neurons (Davies et al., 1986; Ernfors et al., 1990;

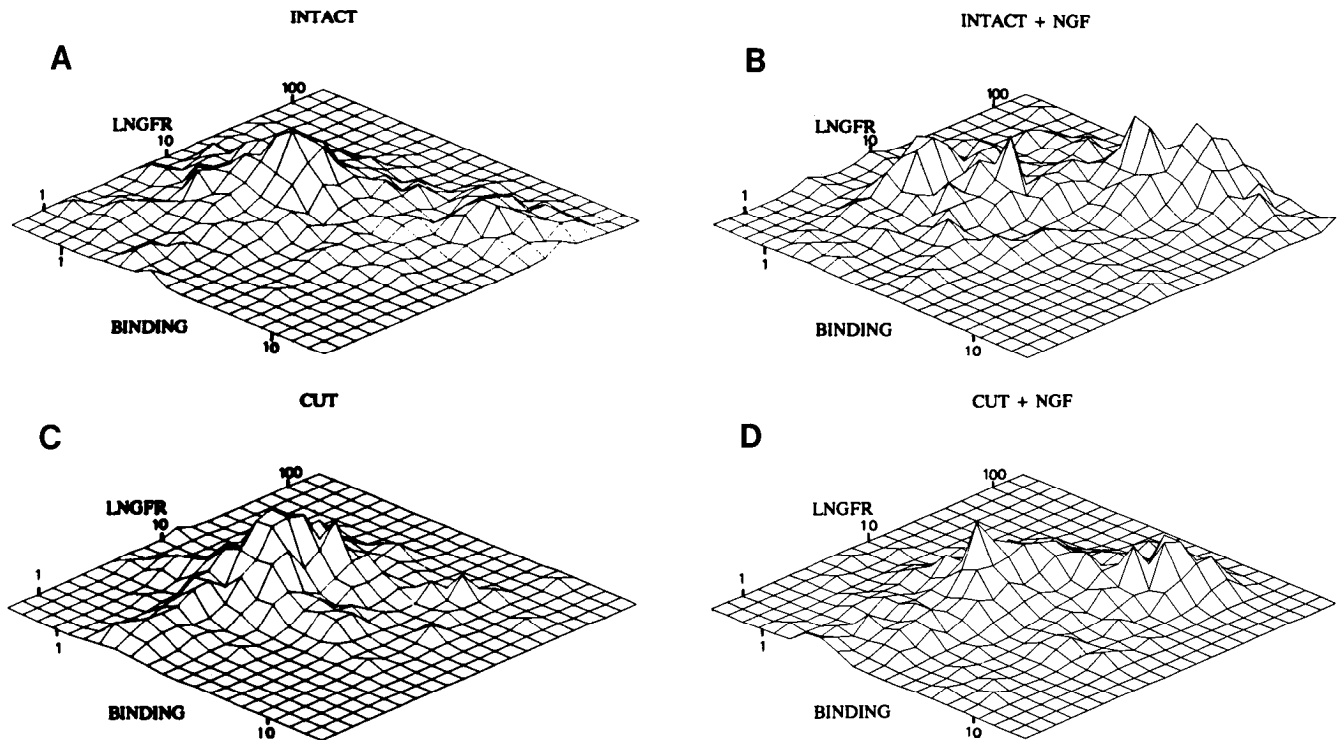


Figure 8. Two weeks after right sciatic nerve transection, rats were perfused intrathecally for 1 week with NGF or control solution. Three-dimensional frequency histograms characterize labeling with the LNGFR probes and ^{125}I -NGF. For neurons with high-affinity receptors (heavily labeled with ^{125}I -NGF), NGF infusion induces LNGFR mRNA above normal concentrations in uninjured neurons (*B*) and counteracts the downregulation of LNGFR mRNA in axotomized neurons (*D*). Neurons lacking high-affinity receptors (lightly labeled by ^{125}I -NGF) are not appreciably influenced by NGF.

Hohn et al., 1990; Maisonpierre et al., 1990). Although the density of LNGFR mRNA labeling varies among sensory neurons, the single normal distribution in frequency histograms suggests that all mature sensory neurons have retained the capacity to express the LNGFR gene. The fact that all or almost all sensory neurons with high-affinity NGF receptors display relatively high concentrations of LNGFR mRNA could be explained by two hypotheses, not mutually exclusive. LNGFR could be present in NGF-responsive cells as an essential component of the functional NGF receptor and/or a result of induction by endogenous NGF.

The data on rat sympathetic and primary sensory neurons are consistent with tissue culture evidence on pheochromocytoma cells (Hempstead et al., 1991; Kaplan et al., 1991a,b; Klein et al., 1991a; Loeb et al., 1991; Radeke and Feinstein, 1991) that *trk* is a necessary constituent of the functional NGF receptor.

In quantitative colocalization data in lumbar DRG (Fig. 2) and observations on trigeminal ganglia, it is concluded that neurons have high-affinity binding sites if and only if they have abundant *trk* mRNA. The underlying assumption that ^{125}I -NGF labeling can be used to determine the presence or absence of high-affinity NGF receptors has been substantiated in previous studies of rat lumbar DRG (Verge et al., 1989a). In nodose ganglia, all neurons with high concentration of *trk* mRNA have many NGF-binding sites but some neurons moderately labeled with ^{125}I -NGF have little or no *trk* mRNA. We suspect that neurons in this latter subpopulation have relatively abundant low-affinity binding without high-affinity binding as do Schwann cells in the distal segment of a transected nerve (Richardson et al., 1989). The less plausible explanation, that high-affinity NGF

binding is present on some nodose neurons without *trk*, is technically difficult to exclude.

Infusion of NGF strongly upregulates LNGFR mRNA in both uninjured and injured primary sensory neurons. The effects of infused NGF on LNGFR mRNA are apparent only in the subpopulation of sensory neurons that have high-affinity NGF binding sites. Here, NGF may have an important function in regulating the synthesis of LNGFR as well as that of several neuropeptides (Goedert et al., 1981; Schwartz et al., 1982; Fitzgerald et al., 1985; Lindsay and Harmar, 1989; Verge et al., 1991), neurofilament (Verge et al., 1990b), and the growth-associated protein GAP-43 (Verge et al., 1990a). NGF has been shown previously to induce LNGFR mRNA and/or protein in basal forebrain cholinergic neurons (Cavicchioli et al., 1989; Hagg et al., 1989), sympathetic and sensory neurons (Lindsay et al., 1990; Miller et al., 1991), and pheochromocytoma cells (Bernd and Greene, 1984). In pheochromocytoma cells, this regulation is at the transcriptional level (Miller et al., 1991). Other agents that have been reported to influence expression of the LNGFR gene in diverse cell types are basic fibroblast growth factor (Birren and Anderson, 1990; Tajiri et al., 1992) and ciliary neurotrophic factor (Hagg et al., 1992).

Exogenous NGF has no demonstrable effect on *trk* mRNA in uninjured sensory neurons but counteracts axotomy-induced downregulation of *trk* mRNA in the same neuronal population. The finding that NGF can influence expression of the *trk* gene, not predicted from tissue culture studies on PC12 pheochromocytoma cells (Kaplan et al., 1991a), could reflect either transcriptional or posttranscriptional regulation and could be direct or indirect. Only fragmentary information is now available con-

cerning the actions of NGF, retinoic acid (Rodriguez-Tébar and Rohrer, 1991), and unidentified molecules in conferring the cell-specific capacity to express the *trk* gene or modulating its expression. One possible explanation for the different effects of NGF infusion on *trk* mRNA in uninjured and injured neurons is that availability of endogenous NGF is a limiting factor in expression of the *trk* gene after axotomy but not under normal circumstances. Alternatively, NGF may induce *trk* mRNA only in the presence of some additional factor that is activated in neurons by axonal injury.

trk mRNA and high-affinity NGF-binding sites appear to undergo parallel changes after axotomy and NGF infusion to the extent that measurements of an mRNA and protein can be compared. Although the changing concentrations of high-affinity receptors could entirely reflect changes in *trk* mRNA, the possibility cannot be excluded that the changes in LNGFR are contributory. Neither the normal data nor changes after axotomy or NGF infusion clearly discriminate whether or not LNGFR is necessary for functional high-affinity NGF receptors.

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