19. Pairwise short tandem repeat (STR)–based [S. Henikoff, *New Biol.* **3**, 1148 (1991)] similarity scores were calculated and used as a basis for the phenograms. For a set of sequences representing a diverse panel of peptides used for a peptide-based enzyme-linked immunosorbent assay [A. Pau *et al.*, *AIDS Res. Hum. Retroviruses* **11**, 1369 (1994)], clusters generated by means of phenograms were highly correlated with the serological typing results (B. T. M. Korber, unpublished results).
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21. The entropy, H_i, is defined in terms of the probabilities, Ps, of the different protein forms, s, that can appear at a given time point i (for example, we consider the symbols $s = A, B, C, \ldots$ to represent the protein clusters defined in Fig. 2). H_i is defined as $H_i = -\sum_{s=A,B,C,\ldots} Ps_i \times \log Ps_i$. This measure, also known as the Shannon entropy, is related to the Simpson index that has been used previously to quantify viral sequence variability within time points (*10*), as both are specific forms of Renyi entropies [A. Renyi, *Probability Theory* (North-Holland, Amsterdam, 1970); P. Grassberger, *Phys. Lett.* **A97**, 227 (1983)]. These analyses correlated with the within-time point amino acid Hamming distances, after gap-stripping.
22. The association of low genetic diversity with a rapid rate of progression to AIDS has been replicated and further substantiated by the results from two natural history cohort studies of HIV-1–infected men based on the heteroduplex mobility assay (E. Delwart *et al.*, in preparation; S. Wolinsky, unpublished results) and by the results of a natural history cohort study of children with perinatal HIV-1 infection (S. Ganeshan *et al.*, in preparation).
23. Neutralization studies were conducted by testing an early and a late isolate against a panel of early sera and late sera from each of the six subjects. Consistent with the observation that a neutralizing antibody response develops gradually [J. P. Moore, Y. Cao, D. D. Ho, R. A. Koup, *J. Virol.* **68**, 5142 (1994)], most of the sera obtained from these participants at an earlier time point had little or no neutralizing activity. The geometric mean titer of the reciprocal dilution of early sera needed to neutralize 90% of an autologous or heterologous virus inoculum (no neutralization was arbitrarily set at 0.01) for all six subjects ranged from 0.03 to 0.06. The geometric mean titers of the reciprocal dilution of late sera needed to neutralize 90% of a virus inoculum were 0.03 for P1, 0.2 for P2, 5.6 for P3, 7.9 for P4, 1.8 for P5, and 0.2 for P6. Therefore, we did not observe a clear association between neutralization capability and the rate of progression to disease. Additionally, the viruses isolated early and late from P1 and the virus isolated late from P2 were the only viruses neutralized by early serum and were generally the most easily neutralized viruses isolated. Mean reciprocal titers of 28.5 and 22.6 were observed for early and late viruses isolated from P1, respectively, and 5.3 for a late virus isolated from P2. The viruses isolated from the blood obtained from the other four subjects had mean reciprocal titers ranging from 0.01 to 3.7. Thus, the propagated viruses from rapid progressors P1 and P2 may be intrinsically easier to neutralize.
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27. To evaluate the biological phenotype of the viruses isolated from each subject, we tested four to six separate first passage virus culture stocks covering the course of infection for their capacity to replicate in primary cultures of normal donor peripheral blood mononuclear cells and monocyte-derived macrophages and for their syncytium-inducing properties in cultures of the established MT-2–transformed T cell line. The isolated viruses were expanded once in normal donor peripheral blood mononuclear cells, and titers were determined for infectivity as described [R. I. Connor, H. Mohri, Y. Cao, D. D. Ho, *J. Virol.* **67**, 1772 (1993)].
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Selective Activation of NF-κB by Nerve Growth Factor Through the Neurotrophin Receptor p75

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Nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 (NT-3) selectively bind to distinct members of the Trk family of tyrosine kinase receptors, but all three bind with similar affinities to the neurotrophin receptor p75 ($p75^{NTR}$). The biological significance of neurotrophin binding to $p75^{NTR}$ in cells that also express Trk receptors has been difficult to ascertain. In the absence of TrkA, NGF binding to $p75^{NTR}$ activated the transcription factor nuclear factor kappa B (NF-κB) in rat Schwann cells. This activation was not observed in Schwann cells isolated from mice that lacked $p75^{NTR}$. The effect was selective for NGF; NF-κB was not activated by BDNF or NT-3.

The best established role for neurotrophins, which include NGF, BDNF, NT-3, NT-4/5, and NT-6, is their ability to support the survival and differentiation of neurons. Three tyrosine kinase receptors, referred to as theTrks, are critically involved in mediating these effects (*1*). However, the neurotrophins also interact with another receptor whose function has not been clearly established, $p75^{NTR}$. The Trks exhibit high selectivity for their cognate ligands (TrkA for NGF, TrkB for BDNF and NT-4/5, and TrkC for NT-3), whereas $p75^{NTR}$ binds to all neurotrophins with similar affinity but with different kinetics (*2*). The Trks produce their effects through activation of well-described signaling pathways, including Ras–mitogen-activated protein kinase, phospholipase C–γ, and phosphoinositol-3-OH kinase (*3*). On the other hand, the transduction mechanisms of $p75^{NTR}$ remain largely unknown. Several reports suggest that $p75^{NTR}$ interacts with TrkA to form a high-affinity binding site (*4*) and to regulate TrkA signaling (*5*). However, evidence has also been presented that suggests that $p75^{NTR}$ has an independent function, such as the regulation of apoptosis (*6*) or the migration of Schwann cells (*7*). The underlying transduction mechanisms have yet to be determined. Recently, neurotrophin binding to $p75^{NTR}$ was shown to activate the spingomyelinase

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pathway in a glioma cell line and in transfected NIH 3T3 cells to produce the lipid messenger ceramide (*8*). This pathway is used by a number of cytokine receptors, including the tumor necrosis factor (TNF) receptor 1, which has sequence homology to $p75^{NTR}$ and has been shown to be directly involved in mediating cell death after TNF binding (*9*). One of the downstream targets activated by the TNF receptor 1 is the transcription factor NF-κB, which, when activated by external stimuli, translocates to the nucleus where it binds to DNA and regulates gene transcription (*10*).

Because neurotrophins can activate ceramide production in transformed cells, we decided to investigate whether neurotrophin binding to $p75^{NTR}$ could activate NF-κB in Schwann cells isolated from the rat sciatic nerve. In the axotomized sciatic nerve there is a marked up-regulation of $p75^{NTR}$ in Schwann cells distal to the lesion (*11*), which suggests a potential involvement of $p75^{NTR}$ in regeneration. We used a monoclonal antibody raised to an epitope on the p65 subunit of NF-κB (also referred to as RelA), which is normally inaccessible because of binding of the endogenous inhibitor of NF-κB, IκB (*10*). The antigenic site is exposed upon activation of the pathway leading to degradation of the inhibitor, followed by subsequent nuclear translocation.

Schwann cells prepared from the sciatic nerve of postnatal day 2 (P2) rats were cultured overnight, then treated with NGF (100 ng/ml) for various periods of time, fixed, and immunostained with the antibody to p65 (Fig. 1A). NGF promoted a strong nuclear staining that could be seen within 30 min and lasted at least 4 hours. Because Schwann cells produce substantial quantities of NGF-like material themselves, it proved critical to culture them at low density (corresponding to 4×10^5 to 8×10^5 cells on a plate 10 cm in diameter) in order to avoid autoactivation.

$p75^{NTR}$ binds to NGF, BDNF, and NT-3 with similar affinity and all are capable of activating the ceramide pathway in NIH 3T3 cells transfected with this receptor (*12*). To determine if they could also all activate NF-κB, each was added to the Schwann cell cultures and the cells were stained for the p65 subunit of NF-κB. Only NGF proved capable of activating this transcription factor. Neither BDNF nor NT-3 showed any stimulation (Fig. 1A). Because the NGF effect was specific despite the ability of the other neurotrophins to bind to $p75^{NTR}$, we checked to see if the effects seen under our conditions could be due to TrkA by performing reverse transcription and polymerase chain reaction (PCR) on RNA isolated from these cultures (*13*). No TrkA signal was detectable in these cultures after 40 cycles of amplification, confirming previous reports indicating that these cells did not express this tyrosine kinase receptor (*14*).

To further verify that $p75^{NTR}$ was mediating the effects of NGF, we repeated the experiments using Schwann cells isolated from the sciatic nerve of mice in which the gene encoding $p75^{NTR}$ had been deleted (*15*). In these cells, NGF did not induce NF-κB nuclear staining, which indicates that $p75^{NTR}$ is necessary for activation (Fig. 1B).

In addition to nuclear translocation, activation of NF-κB enables it to bind to specific DNA sequences. To demonstrate that NGF-activated NF-κB was capable of binding to DNA, we performed electrophoretic mobility shift assays (EMSAs) using a double-stranded oligonucleotide containing an NF-κB consensus sequence from the murine κ light-chain enhancer. The Schwann cells were treated with the neurotrophins, and the total cell extracts were tested for the ability to bind the DNA. Cells treated for 1 to 2 hours showed a concentration dependence that indicated half-maximal stimulation with NGF at about 30 ng/ml (Fig. 2). This concentration roughly corresponds to the dissociation constant for NGF binding to $p75^{NTR}$, as opposed to that observed in cells expressing TrkA in addition to $p75^{NTR}$ (*4*). Neither BDNF nor NT-3 was capable of

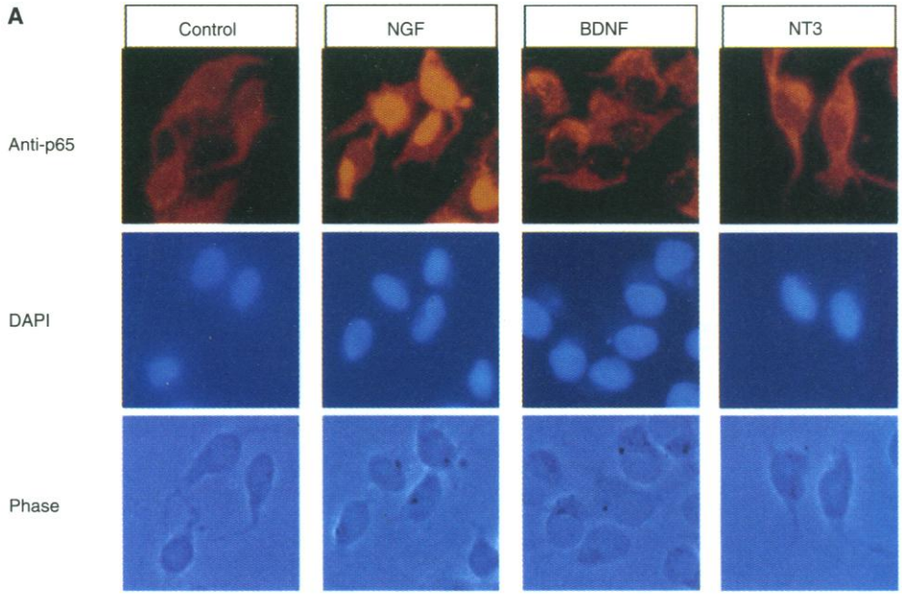

Fig. 1. Nuclear translocation of NF-κB is stimulated by NGF but not by BDNF or NT-3 in Schwann cells. (**A**) Cells were prepared from P2 rat sciatic nerves as described previously (*23*) and plated at low density on chamber slides in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. They were identified as Schwann cells by staining with an antibody raised against the marker protein S100 (Sigma) (*17*), cultured overnight, and treated with recombinant neurotrophin (NGF, BDNF, or NT-3; 100 ng/ml) for 2 hours or not treated (control). The cells were fixed with methanol and analyzed by indirect immunofluorescence with a monoclonal antibody specific for the activated subunit of NF-κB, p65 (*24*) (upper panels). The same cells were stained for nuclear DNA with 4,6-diamidino-2-phenylindole (DAPI) (middle panels) and the lower panels show phase contrast images. (**B**) Schwann cells prepared from the sciatic nerve of P2 mice with the gene encoding $p75^{NTR}$ deleted (*15*) were cultured overnight, treated with NGF (100 ng/ml), and fixed and stained as in (A).

activating NF-κB.

Because neurotrophin binding to $p75^{NTR}$ results in the production of ceramide (*12*), we tested whether this lipid second messenger could also activate NF-κB. Following a 1- to 2-hour exposure to 1 μM C2-ceramide, NF-κB in Schwann cells was activated, as evidenced by DNA binding of cell extracts (Fig. 2).

The presence of neurotrophin-binding proteins other than $p75^{NTR}$ in Schwann cells, such as truncated TrkB and TrkC (*14*), made it important to determine whether $p75^{NTR}$ alone would be sufficient to mediate the observed effects. Therefore, we used mouse PCNA cells, which are L cell derivatives that express this receptor (*16*). The parental L cells do not bind BDNF or NT-3 (*2*). NGF activated NF-κB in these cells with a concentration dependence similar to that observed in the Schwann cells (Fig. 3A). The activation occurred within 10 min and was maintained for at least 5 hours (*17*). $p75^{NTR}$ was clearly sufficient for mediation of these effects, because no activation of NF-κB by NGF was observed in the parental L cells. As with Schwann cells, neurotrophin activation of NF-κB in PCNA cells was specific for NGF, as BDNF (Fig. 3B) and NT-3 (*17*) failed to elicit a response. As a control, extracts were prepared from PCNA cells after treatment with the phorbol ester phorbol 12-myristate 13-acetate (PMA), which is known to activate NF-κB in many cells (*10*) (Fig. 3B).

There are currently five DNA binding proteins known that can potentially heterodimerize to form the transcription factor NF-κB, the most common of which are the p65 (or RelA) and p50 subunits (*10*). To identify the subunits activated by NGF in PCNA cells, we performed supershift assays in which the cell lysates were preincubated with specific antibodies to p65, c-Rel, and p50. The antibodies shifted the band on the EMSA, thus demonstrating that all three proteins are present in the complexes activated by NGF in the PCNA cells (Fig. 3C). Because of the difference in binding kinetics between NGF and, in particular, BDNF to $p75^{NTR}$ (*2*), we tested the possibility that BDNF activation of NF-κB could require a longer time to be detected. However, even after 5 hours of BDNF treatment, no activity over basal activity was detected. Another possibility to account for the difference between NGF and the two other neurotrophins could be the presence of other molecules with which BDNF or NT-3 interacts. Indeed, it has been shown that TrkA exerts an inhibitory activity on $p75^{NTR}$-mediated ceramide production (*12*), which could explain the very slow time course of NF-κB induction by NGF in PC12 cells (*18*). However, the parental L cells do not to bind to BDNF or NT-3, indicating that receptors for these neurotrophins (such as TrkB or TrkC) are not expressed in these cells (*2*). Thus, only NGF produces a signal through $p75^{NTR}$, which results in the activation of the transcription factor NF-κB. NGF may preferentially activate additional, yet-to-be-determined pathways through $p75^{NTR}$ that, in collaboration with ceramide, activate this transcription factor.

These results provide evidence for a NGF-specific, TrkA-independent, signal transduction mechanism for $p75^{NTR}$ in nontransformed cells that results in the activation of the transcription factor NF-κB. This potent regulator of gene expression activates the expression of various cell surface molecules (*10*), and previous studies with Schwann cells and PC12 cells have demonstrated that NGF can up-regulate the adhesion glycoprotein L1 in the absence of TrkA activation (*19*). Thus,

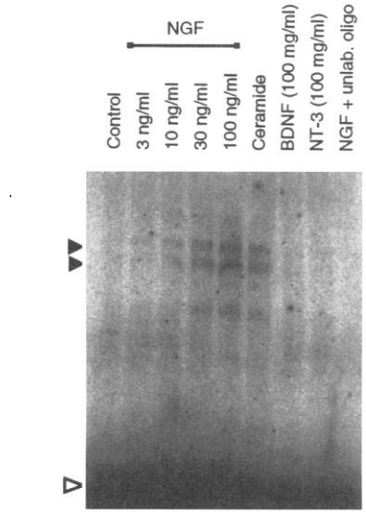

Fig. 2. Neurotrophin effects on DNA binding activity of NF-κB in Schwann cells. Schwann cells cultured overnight were not treated (control) or were treated for 1 hour with NGF (3, 10, 30, or 100 ng/ml), 1 μM ceramide, or BDNF or NT-3 (100 ng/ml), then lysed in 20 mM Hepes (pH 8.0), 0.35 M NaCl, 20% glycerol, 1% NP-40, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM EGTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. The lysates were incubated with a ^{32}P-labeled oligonucleotide corresponding to a sequence of the murine κ light-chain enhancer as described (*25*) for 30 min on ice. The mixtures were then separated on a 4% nondenaturing acrylamide gel, which was then dried and analyzed with a Fuji Phosphoimager. The specific band seen after NGF treatment could be eliminated by competition with an unlabeled (unlab.) oligonucleotide. The positions of immunoreactive complexes (solid arrowheads) and the uncomplexed DNA (open arrowhead) are indicated.

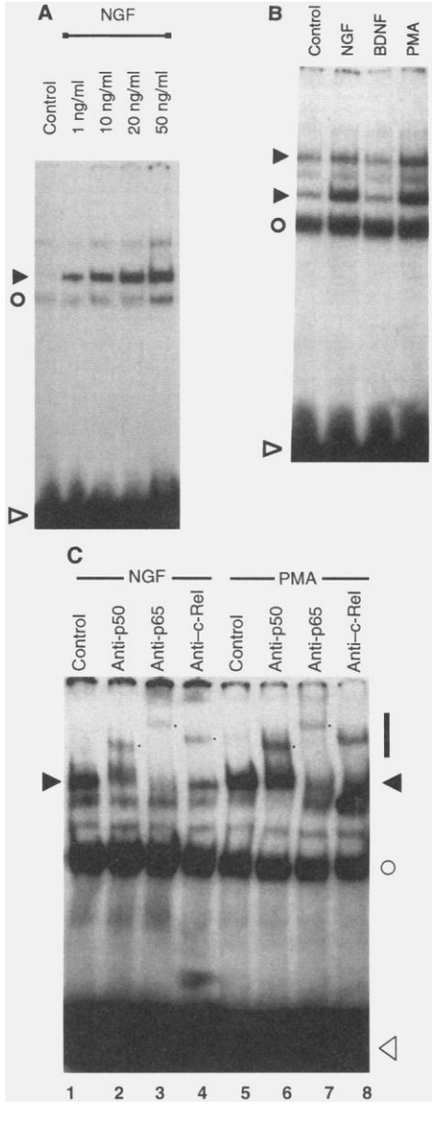

Fig. 3. Neurotrophin effects on DNA binding activity of NF-κB in PCNA cells. (**A**) PCNA cells were not treated (control) or were treated with recombinant NGF (1, 10, 20, or 50 ng/ml) for 2 hours. (**B**) Cells were not treated (control) or were treated with NGF, BDNF, or NT-3 (100 ng/ml) (*17*) or with PMA (50 ng/ml) for 2 hours, and cell lysates were analyzed for DNA binding activity as described for Fig. 2. The positions of immunoreactive complexes (solid arrowheads), the uncomplexed DNA (open arrowhead), and nonspecific bands (circle) are indicated. The additional band (above arrowhead; see also Fig. 2) is presumably due to complexes of p65 and c-Rel [see (*26*)]. (**C**) Bands were identified as complexes of p50, p65, and c-Rel by addition of each antibody separately to the DNA binding reaction of cell lysates from the PCNA cells treated with NGF (50 ng/ml) or PMA (50 ng/ml) (control denotes the binding reaction without addition of antibody). The bound oligonucleotide was separated from the free oligonucleotide as described in Fig. 2. The positions of immunoreactive complexes (solid arrowheads), the uncomplexed DNA (open arrowhead), nonspecific bands (circle), and the supershifted bands (vertical bar) are indicated. Some supershifted bands (marked with dots) are very faint because the binding of the antibodies to NF-κB complexes competed with their ability to bind DNA [see (*25*)]. No activation of NF-κB was observed after exposure of L cells to NGF.

NGF activation of NF-κB through $p75^{NTR}$ may up-regulate the expression of such extracellular matrix proteins in Schwann cells, thereby influencing their migration during nerve regeneration (*7*).

The NGF–$p75^{NTR}$–NF-κB signaling pathway may also play a role in other pathophysiological states. NGF is, so far, unique among the neurotrophins in acting as a link between inflammation and the peripheral nervous system (*20*). NGF levels are up-regulated in inflamed tissue (*21*), and it has been shown that NGF is released by cells of the immune system (*20*), as are cytokines, which act through NF-κB. NGF is also known to be required for the hyperalgesia accompanying tissue damage, and it exerts its effects on nociceptive sensory neurons (*22*). Thus, it can be envisaged that NGF activates the $p75^{NTR}$–NF-κB pathway in a context relevant to the generation of hyperalgesia.

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Cerebellum Implicated in Sensory Acquisition and Discrimination Rather Than Motor Control

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Recent evidence that the cerebellum is involved in perception and cognition challenges the prevailing view that its primary function is fine motor control. A new alternative hypothesis is that the lateral cerebellum is not activated by the control of movement per se, but is strongly engaged during the acquisition and discrimination of sensory information. Magnetic resonance imaging of the lateral cerebellar output (dentate) nucleus during passive and active sensory tasks confirmed this hypothesis. These findings suggest that the lateral cerebellum may be active during motor, perceptual, and cognitive performances specifically because of the requirement to process sensory data.

For a century, the cerebellum has been regarded as a motor organ (*1*). Lesions to the cerebellum cause incoordinated movement (*2*), and the cerebellum is activated during movement (*3*, *4*). Recent studies of brain-injured humans revealed that the cerebellum is instrumental in nonmotor behaviors such as judging the timing of events, solving perceptual and spatial reasoning problems, and generating words according to a semantic rule (*5*). Very recently, cerebellar activity has been detected during these perceptual and cognitive behaviors (*6*) and during the mental rotation of abstract objects (*7*). Such findings challenge classical motor theories of cerebellar function. Although the cerebellum receives input from virtually every sensory system (*8*, *9*) and is activated by tactile stimulation alone (without movement) (*3*), it has not been considered a sensory organ because cerebellar lesions do not cause gross sensory deficits (*2*). However, ascertaining whether neural tissue has a motor or sensory function is a subtle problem because motor behavior is guided by ongoing sensory acquisition of object information, and motor efficiency (the accuracy, coordination, and smoothness of motor behavior) depends on continuously updated sensory data.

To dissociate sensory acquisition and discrimination from motor performance per se, we imaged blood oxygenation change, a correlate of neural activity, in the lateral (dentate) nucleus of humans as they performed tasks involving passive and active sensory discriminations. The dentate nucleus is the sole output for the large lateral hemispheres of the primate cerebellum, and its activation has usually been linked to finger movements (*10*). We tested the hypothesis (*11*) that dentate activation is more closely associated with sensory discriminations made through the fingers than with finger-movement control per se.

Six healthy volunteers performed four tasks (*12*). In the Cutaneous Stimulation (CS) task (*13*), they passively experienced sandpaper rubbed against the immobilized pads of the second, third, and fourth fingers of each hand. In the Cutaneous Discrimination (CD) task (*13*), they were asked to actively compare (without responding) whether the coarseness of the sandpaper on the two hands matched. The coarseness of the sandpaper changed randomly every 3 s. In the Grasp Objects (GO) task (*14*), they used each hand to repeatedly reach for, grasp, raise, and then drop an object. In the Grasped Objects Discrimination (GOD) task (*14*), they grasped one object with one hand while using the

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