4. L. Edwall and L. Olgart, *Pain* **3**, 121 (1977); M. Ahlquist, L. Edwall, O. Franzen, G. Haegerstam, *ibid.* **19**, 353 (1984); U. Fors, L. Edwall, G. Haegerstam, *ibid.* **33**, 253 (1988). But also see W. K. Dong, E. H. Chudler, and R. F. Martin [*Brain Res.* **334**, 389 (1985)] for evidence of a small population of nonnociceptive neurons in cat tooth pulp.
5. A crystal of $DiIC_{18}$ (Molecular Probes, Eugene, OR) was placed in maxillary molars of male Sprague-Dawley rats (200 to 250 g) largely as described in J. L. Stephenson and M. R. Byers [*Exp. Neurol.* **131**, 11 (1995)] and in C. F. Marfurt and D. F. Turner [*J. Comp. Neurol.* **223**, 353 (1984)]. However, we drilled the crown only until a change in resistance was detected; this conservative procedure exposes the tip of the pulp chamber and results in no evident bleeding. Trigeminal ganglia were dissected 2 to 5 days after placing the dye and were dissociated as described (*16*) except for an additional centrifugation through 30% Percoll (Sigma) to remove axon debris. After dissection, the region near the tooth was checked for dye leakage. Dye leakage disqualified a preparation from use, which in fact did not occur once we became proficient with the method. Dispersed cells were resuspended in F12 growth medium, 10% fetal calf serum, and 50 ng/ml of nerve growth factor (2.5S) (Biomedical Technologies, Stroughton, MA), and plated onto glass cover slips coated with poly-D-lysine and laminin. After several hours at 37°C in 5% CO_2, cultures were transferred to L15 media (without nerve growth factor) and maintained at 4°C in air to inhibit neurite outgrowth. All cells were studied within 48 hours of dissociation. Results with freshly dissociated cells were indistinguishable from results with cells maintained in culture, which indicates that properties did not change with these culture times and conditions.
6. Sodium in the extracellular solution greatly diminished rundown of I_{Ca}. Typically, base-line I_{Ca} in neurons of all sizes was very stable for 15 min after an initial few minutes of variability (see, for example, Fig. 3B); such stability greatly facilitated comparison of modulatory effects in the cell populations. Current through Na^+ channels was not fully blocked by either tetrodotoxin or saxitoxin, so I_{Ca} amplitude was measured at least 50 ms after the onset of the pulse, when Na^+ channels were fully inactivated. A Ca^{2+} channel blocker (1 mM Cd^{2+}) applied at the end of most of the experiments confirmed that sustained current passed only through Ca^{2+} channels. The percentage of inhibition was determined by comparing I_{Ca} amplitude before drug application to amplitude 40 s after application. Solutions flowed over the cell at all times and were exchanged within seconds with an array of 1-μl pipettes. Extracellular solution consisted of 135 mM NaCl, 5 mM KCl, 1 mM MgCl, 5 mM $CaCl_2$, 10 mM Hepes, and 10 mM glucose (pH 7.3). Whole-cell pipette (intracellular) solution consisted of 100 mM CsCl, 1 mM sodium adenosine triphosphate, 0.3 mM guanosine triphosphate, 10 mM EGTA, 2.5 mM $MgCl_2$, 2 mM $CaCl_2$, 8.8 mM sodium phosphocreatine, 0.08 mM leupeptin, and 40 mM Hepes (pH 7.0) (titrated with tetraethylammonium hydroxide).
7. J. Sandkuhler, Q.-G. Fu, C. Helmchen, *Neuroscience* **34**, 565 (1990).
8. T. J. Wilding, M. D. Womack, E. W. McCleskey, *J. Neurosci.* **15**, 4124 (1995).
9. B. Y. Cooper, C. J. Vierck Jr., D. C. Yeomans, *Pain* **24**, 93 (1986); I. Jurna and G. Heinz, *Brain Res.* **171**, 573 (1979).
10. T. Lewis and E. E. Pochin, *Clin. Sci.* **3**, 67 (1937); H. Torebjork and R. G. Hallin, *Exp. Brain Res.* **16**, 321 (1972).
11. E. Jyvasjarvi and K.-D. Kniffki, *J. Physiol. (London)* **391**, 193 (1987); M. L. Ahlquist *et al.*, in *Advances in Pain Research and Therapy*, H. L. Fields, Ed. (Raven Press, New York, 1985), vol. 9, pp. 352–359.
12. A. A. Harper and S. N. Lawson, *J. Physiol. (London)* **359**, 31 (1985); K. H. Lee, K. Chung, J. M. Chung, R. E. Coggeshall, *J. Comp. Neurol.* **243**, 335 (1986).
13. A. I. Basbaum and H. L. Fields, *Annu. Rev. Neurosci.* **7**, 309 (1984).
14. T. J. Grudt and J. T. Williams, *J. Neurosci.* **14**, 1646 (1994).
15. S. A. Shefner, R. A. North, R. S. Zukin, *Brain Res.* **221**, 109 (1981); J. T. Williams and W. Zieglgansberger, *Neurosci. Lett.* **21**, 211 (1981).
16. P. T. Akins and E. W. McCleskey, *Neuroscience* **56**, 759 (1993).
17. F. A. Dodge and R. Rahamimoff, *J. Physiol. (London)* **193**, 419 (1967); R. A. Goda and C. F. Stevens, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12942 (1994).
18. M. R. Byers, *Int. Rev. Neurobiol.* **25**, 39 (1984); M. Narhi, H. Yamamoto, D. Ngassapa, T. Hirvonen, *Arch. Oral Biol.* **39**, 23S (1994).
19. E. Seward, C. Hammond, G. Henderson, *Proc. R. Soc. London Ser. B Biol. Sci.* **244**, 129 (1991); J. E. Schroeder, P. S. Fischbach, D. Zheng, E. W. McCleskey, *Neuron* **6**, 13 (1991); H. C. Moises, K. I. Ruskin, R. L. Macdonald, *J. Neurosci.* **14**, 5903 (1994).
20. We thank M. Byers, R. Goodman, A. Roos, and J. Williams for helpful critiques of an earlier version of the manuscript. Supported by the National Institute of Drug Abuse and the Lucille P. Markey Charitable Trust. A.T. received a Porter Fellowship (American Physiological Society) and participated in the Washington University–Howard Hughes Medical Institute undergraduate research program. E.W.M. received a Presidential Young Investigator Award from NSF.

16 June 1995; accepted 1 September 1995

Regulated Expression of the Neural Cell Adhesion Molecule L1 by Specific Patterns of Neural Impulses

Kouichi Itoh, Beth Stevens, Melitta Schachner, R. Douglas Fields*

Development of the mammalian nervous system is regulated by neural impulse activity, but the molecular mechanisms are not well understood. If cell recognition molecules [for example, L1 and the neural cell adhesion molecule (NCAM)] were influenced by specific patterns of impulse activity, cell-cell interactions controlling nervous system structure could be regulated by nervous system function at critical stages of development. Low-frequency electrical pulses delivered to mouse sensory neurons in culture (0.1 hertz for 5 days) down-regulated expression of L1 messenger RNA and protein (but not NCAM). Fasciculation of neurites, adhesion of neuroblastoma cells, and the number of Schwann cells on neurites was reduced after 0.1-hertz stimulation, but higher frequencies or stimulation after synaptogenesis were without effect.

Developing before the establishment of synapses, spontaneous electrical activity in prenatal neurons is of undetermined importance (*1*). Evidence suggests that impulses can regulate neuronal differentiation, but the molecular mechanisms are unknown (*1–3*). The neural cell adhesion molecule L1 has a major influence on morphogenesis of the nervous system, by regulating cell adhesion, neurite outgrowth, fasciculation, myelination, and transmembrane signaling (*4*). Possible control of L1 expression by patterned impulse activity was explored in mouse dorsal root ganglion (DRG) neurons maintained in a multicompartment cell culture preparation (Fig. 1A) (*5*), designed to deliver electrical stimulation in patterns resembling the normal impulse activity in developing sensory neurons (*1*).

Stimulation at a rate of one action potential every 10 s (0.1 Hz) reduced L1 mRNA by a factor of 12.7 ± 1.46 after 5 days of stimulation ($P < 0.001$; $n = 8$), as measured by quantitative polymerase chain reaction (PCR) (Fig. 1, C through E). Compared to 0-Hz stimulation ($n = 6$), differences were not significantly lower after 1 or 3 days of stimulation at 0.1 Hz (79 ± 11%, $n = 3$; and 89 ± 5.6%, $n = 3$). Alternative PCR strategies, with neuron-specific enolase ($n = 4$) or cyclophilin ($n = 6$) for normalization, or coamplification with the internal L1 mimic ($n = 27$) (Fig. 1B), confirmed the reduction after 5 days.

We next investigated whether regulation of L1 mRNA was sensitive to the particular pattern of impulses, as would be required to optimize structural and functional relations according to the information processing efficacy of the newly formed circuit. Stimulation at higher frequencies for 5 days (0.3 or 1 Hz; $n = 3$ and 4) (Fig. 1B) and pulsed stimulation (0.5-s, 10-Hz bursts every 2 s; $n = 2$) failed to alter levels of L1 mRNA. Chronic membrane depolarization for 5 days with 60 mM KCl was without effect ($n = 3$).

Protein immunoblot analysis showed a decrease in L1 polypeptide amounts accompanying the reduction of L1 mRNA (Fig. 2). Both molecular species of L1 in DRG neurons (210 and 230 kD) (*6*, *7*) decreased significantly after 5 days of 0.1-Hz stimulation ($P < 0.05$, $n = 21$), but not after an

K. Itoh, B. Stevens, R. D. Fields, National Institutes of Health, National Institute of Child Health and Human Development, LDN 49/5A38, Neurocytology and Physiology Unit, Bethesda, MD 20892, USA.
M. Schachner, Department of Neurobiology, Swiss Federal Institute of Technology, Honggerberg, 8093 Zurich, Switzerland.

*To whom correspondence should be addressed.

equal period of 1-Hz stimulation (n = 15) (Fig. 2). In contrast to L1, none of the three polypeptide bands of NCAM (180, 140, and 120 kD) was influenced by electrical stimulation (Fig. 2; n = 11). Regulation of L1 levels could be mediated by decreased transcription or destabilization of L1 mRNA.

We tested whether the same frequency of stimulation that down-regulated L1 affected essential developmental processes known to be mediated by L1. Adhesion of neuroblastoma (N2a) cells to DRG neurites (Fig. 3, A through C) was reduced significantly after 5 days of 0.1-Hz stimulation (P < 0.002, n = 17) (8) (Fig. 4A) but not after 1-Hz stimulation compared to controls (n = 14) (Fig. 3, A and C, and Fig. 4A). This adhesion assay is largely dependent on homophilic L1-L1 binding, as indicated by inhibition of N2a cell adhesion to DRG axons after preincubation of cells with antibodies against L1, but antibodies against NCAM had no effect (9). When L1 expression was up-regulated by exposure to nerve growth factor (NGF) (50 ng/ml) (*10*) during the 0.1-Hz stimulus period, the reduction in adhesion of N2a cells to stimulated neurites was prevented (n = 6) (Fig. 4A).

These results strongly implicate reduced L1 expression in the stimulus-induced reduction in adhesion, rather than NCAM or calcium-dependent adhesion molecules. The data do not exclude the possibility that other molecules might also be regulated by impulse activity to contribute to these functional effects.

Important developmental consequences would result if association of Schwann cells to axons, which is known to involve homophilic L1-L1 binding (*7*), were affected by impulse activity. Schwann cells secrete neurotrophic factors, myelinate larger diameter DRG axons to enable rapid impulse conduction, and ensheath multiple unmyelinated axons into bundles (*11*). Cultured mouse Schwann cells were plated onto DRG cultures that had been stimulated for 5 days at 0.1, 1, or 0 Hz. Approximately 50% as many Schwann cells were present on DRG neurites after 0.1-Hz stimulation compared with cultures stimulated at 1 Hz (P < 0.008, n = 17) or 0 Hz (P < 0.004, n = 18) (Fig. 4B). Counts were made 4 days after stimulation, indicating that effects persist after substantial Schwann cell proliferation.

Axonal fasciculation is a crucial developmental process for growth cone guidance, innervation, the organization of axons into functional groups in central tracts and peripheral nerves, and in synaptic plasticity (*4*, *12*). Neurites formed smaller and less uniform bundles with more random orientations in cultures stimulated at 0.1 Hz (Fig. 3E) compared with those stimulated at 0 Hz (Fig. 3D) or 1 Hz (Fig. 3F). Automated morphometric analysis confirmed that impulse activity reducing L1 expression also reduced fasciculation (P < 0.03; n = 26) (Fig. 4C).

If similar effects occur in vivo, impulse activity could have significant influences on the developing structure of the nervous system by regulating L1 levels. Changes in prenatal firing patterns of DRG neurons have been correlated with distinct developmental phases (*1*), but before these observations on L1 expression, a molecular mechanism for a possible causal relation has not been available. During the period of neurite outgrowth, DRG neurons are electrically inexcitable. Low-frequency spontaneous activity (<0.5 to 1.5 Hz) begins as the nerve terminals reach the periphery and form a diffuse subepidermal plexus by terminal branching. After sensory end-organs differentiate and neurons form central synapses, impulse activity changes to higher frequency and stimulus-evoked bursts (10 to 20 Hz). The nerve terminals then fasciculate, the subepidermal plexus withdraws, and myelination begins. The terminal branching associated with the commencement of low-frequency impulse activity would be consistent with the lower L1 expression and defasciculation in vitro. Larger amounts of L1 could promote neurite outgrowth in early stages of development (*4*) and promote fasciculation of axon terminals and myelination by Schwann cells after

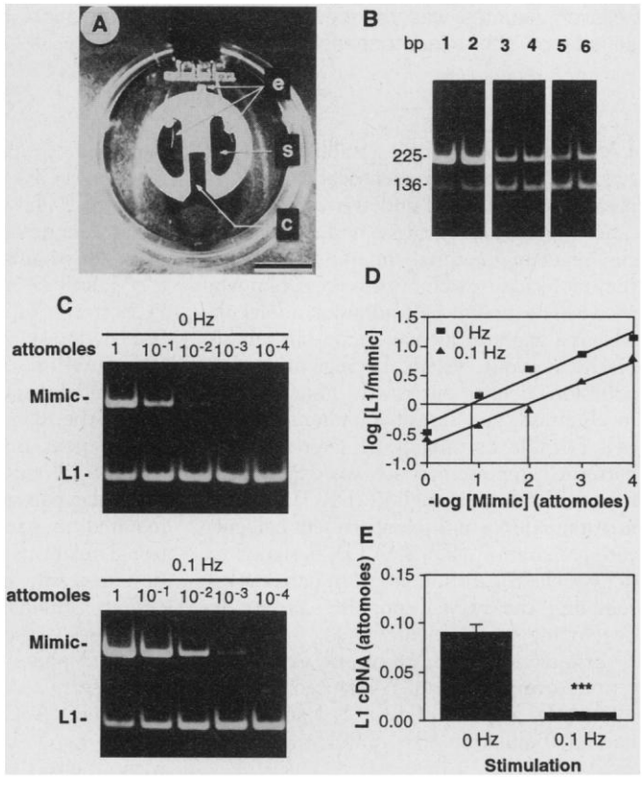


Fig. 1. Regulation of L1 mRNA levels by electrical stimulation in vitro (*14*). (**A**) Mouse DRG neurons were cultured in the side compartments (s) of multicompartment PTFE inserts in 35-mm culture dishes. Neurites grow under the partition between the side and central (c) compartments, allowing low-current stimulation through platinum electrodes (e) on opposite sides of the partitions (*5*). Scale bar = 10 mm. (**B**) Expression of L1 mRNA (136 bp) decreased after 0.1-Hz stimulation for 5 days (lane 1) (P < 0.001, n = 27) but was unchanged after 0.3-Hz (lane 3) (n = 3) or 1-Hz stimulation (lane 5) (n = 3) relative to 0 Hz (lanes 2, 4, and 6) (n = 19). A constant amount (0.1 attomole) of L1 mimic (225 bp) (*15*) was coamplified as an internal standard in each reaction. (**C**) Quantitative PCR analysis with 10-fold serial dilutions of L1 mimic was used to measure concentrations of L1 mRNA after 0-Hz (upper) and 0.1-Hz (lower) stimulation. (**D**) Regressional analysis of the competitive PCR experiment shown in (C). The concentration of L1 mimic yielding an equimolar amplification of endogenous L1 target message and L1 mimic (ratio of logs = 0) is used to quantitate differences in L1 mRNA after stimulation. (**E**) Summary of pooled data from competitive PCR analyses indicating a reduction by a factor of 12.7 ± 1.46 (SEM) in L1 mRNA after 0.1-Hz stimulation (P < 0.001; n = 8). There is no indication that stimulation alters the cellular composition of DRG cultures (*16*). ***P < 0.001.

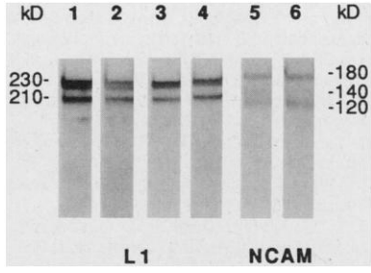


Fig. 2. Effects of electrical stimulation on amounts of L1 and NCAM polypeptide in DRG neurons (*17*). The 230- and 210-kD bands of L1 polypeptide decreased 59 ± 5.3 and 55 ± 4.8%, respectively, after 5 days of stimulation at 0.1 Hz (lane 2) compared with 0 Hz (lane 1) (P < 0.005; n = 21). Stimulation at 1 Hz did not decrease L1 levels (lane 4) compared with 0 Hz (lane 3) [97 ± 2.0 and 104 ± 4.9% of control for the 230- and 210-kD bands, respectively (n = 15)]. In contrast to L1, none of the NCAM polypeptides (120-, 140-, and 180-kD bands) was affected by stimulation at 0.1 Hz (lane 6), compared with 0 Hz (lane 5) (n = 11).

high-frequency impulse activity begins (*1*). Further tests of these correlations in vivo appear warranted.

Consistent with the developmental sequence in utero, we find that the reduction in L1 expression could not be induced by low-frequency stimulation after synaptogenesis with spinal cord neurons. This was shown by plating neurons from the ventral spinal cord into the central compartment of the chamber (*13*). Three weeks later, after DRG neurons in the side compartments had formed abundant synaptic connections with spinal cord neurons (*13*), stimulation at 0.1 Hz for 5 days failed to alter L1 expression in DRG neurons (114 ± 9.3% relative to 0 Hz, $n = 8$).

These studies provide the first evidence that L1 expression is regulated by patterned action-potential stimulation. This activity-dependent regulation is specific to particular temporal patterns of stimulation within the normal physiological range and to specific neural cell adhesion molecules (L1 but not NCAM in these experiments). Major developmental processes, including axon-axon association and Schwann cell–axon interactions, are regulated by the pattern of impulse activity in DRG axons and in accordance with the concomitant changes in L1 expression. These features make regulation of neural cell adhesion molecules by specific patterns of impulse activity an attractive mechanism for controlling cellular interactions that coordinate the structure and function of the nervous system during development and regeneration.

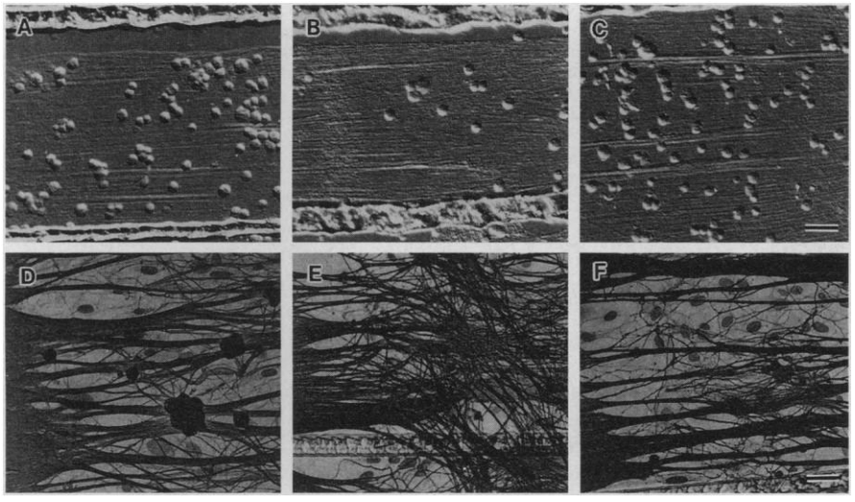
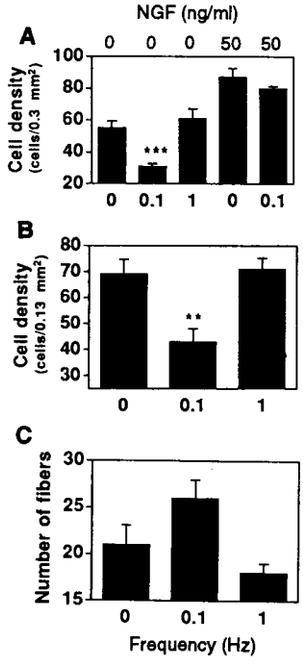


Fig. 3. Electrical stimulation at 0.1 Hz inhibits cell adhesion (*8, 9*) and neurite fasciculation (*18*). (**A** through **C**) DRG axons grow under the partition between central and side compartments within lanes marked by horizontal scratches on the bottom of the culture dish. Removing the insert after the stimulus period provides a uniform compact monolayer of parallel DRG neurites for adhesion assay. Adhesion of N2a cells (*8, 9*) was reduced on neurites stimulated at 0.1 Hz for 5 days (B), compared with unstimulated cultures (A) or cultures stimulated at 1 Hz (C). (**D** through **F**) Fasciculation of DRG neurites in the side compartment was reduced by stimulation after 0.1 Hz for 5 days (E) compared with 0 Hz (D), but was unchanged after stimulation at 1 Hz (F). Scale bar = 50 μm.

Fig. 4. Summary of functional effects of regulating L1 expression by impulse activity in DRG neurons. (**A**) Adhesion of N2a cells was reduced significantly after 5 days of 0.1-Hz stimulation (***$P <$ 0.004 compared with 0 Hz), but adhesion was unchanged after 1-Hz stimulation. Up-regulation of L1 expression in DRG neurites with NGF (50 ng/ml) (*10*) blocked the effects of 0.1-Hz stimulation, suggesting that reduced L1 expression mediated reduced adhesion after 0.1-Hz stimulation (mean and SEM of n = 10, 7, 4, 3, and 3 independent experiments). (**B**) The number of Schwann cells associated with neurites was reduced significantly 4 days after 0.1-Hz stimulation for 5 days (**$P < 0.002$), but Schwann cell numbers were not affected by 1-Hz stimulation for 5 days (n = 9, 9, and 8 experiments) (*19*). (**C**) Fasciculation reduces the number of individual fiber bundles intersecting a linear transect, and the least fasciculation (that is, more fibers) was detected by automated morphometric analysis in cultures stimulated at 0.1 Hz ($P <$ 0.03, n = 26, ANOVA) (*18*).



REFERENCES AND NOTES

1. M. Fitzgerald and B. P. Fulton, in *Sensory Neurons*, S. A. Scott, Ed. (Oxford Univ. Press, New York, 1992), pp. 287–306; M. Fitzgerald, *Nature* **326**, 603 (1987).
2. C. J. Shatz, *Neuron* **5**, 745 (1990); R. D. Fields and P. G. Nelson, *Int. Rev. Neurobiol.* **34**, 133 (1992).
3. G. Xiaonan and N. C. Spitzer, *Nature* **375**, 784 (1995).
4. M. Schachner *et al.*, in *Morphoregulatory Molecules*, G. M. Edelman, B. A. Cunningham, J. P. Thiery, Eds. (Wiley, New York, 1990), pp. 443–468; E. V. Wong, S. Kenwrick, P. Willems, V. Lemmon, *Trends Neurosci.* **18**, 168 (1995).
5. R. D. Fields, C. Yu, E. A. Neale, P. G. Nelson, in *Practical Electrophysiological Methods*, H. Kettenmann and R. Grantyn, Eds. (Wiley-Liss, New York, 1992), pp. 67–76.
6. K. J. Sweadner, *J. Neurosci.* **3**, 2504 (1983).
7. B. Seilheimer and M. Schachner, *J. Cell Biol.* **107**, 341 (1988).
8. After stimulation, the chamber was removed and cultures were washed in Hanks' balanced solution lacking Ca^{2+} and Mg^{2+}, containing 20 mM Hepes and 3 mM EGTA to eliminate calcium-dependent adhesion (for example, cadherins or integrins). The N2a cells were washed and added to DRG cultures for 30 min at 37°C. Cultures were then washed twice with constant agitation for 5 min and fixed with 4% paraformaldehyde. The mean number of N2a cells adhering to neurites in each dish was determined from 10 microscope fields (0.3 mm² per field) in the region under the barrier (n = 26 dishes).
9. The N2a cells were incubated with polyclonal antibodies to mouse L1 or NCAM (200 μg/ml) for 30 min before plating on DRG axons. The L1 antibody reduced adhesion of N2a cells to DRG axons from 55 ± 4.4 to 29 ± 2.0 cells per 0.3 mm² ($P < 0.0005$, n = 12) in unstimulated cultures, and from 31 ± 1.9 to 15 ± 0.95 cells per 0.3 mm² ($P < 0.0003$, n = 9) after 5 days of stimulation at 0.1 Hz. The NCAM antibody had no effect [0 Hz: 55 ± 4.4 versus 68 ± 7.1 cells per 0.3 mm² without and with antibody, not significant (n.s.), n = 12; 0.1 Hz: 31 ± 1.9 versus 28 ± 0.85 cells per 0.3 mm² without and with antibody, n.s., n = 9].
10. K. Itoh, R. Blackenbery, R. A. Akeson, *J. Neurosci* **15**, 2504 (1995).
11. P. Wood *et al.*, *Ann. N.Y. Acad. Sci.* **605**, 1 (1990).
12. C. H. Bailey, M. Chen, F. Keller, E. R. Kandel, *Science* **256**, 645 (1992); U. Rutishauser and L. Landmesser, *Trends Neurosci.* **14**, 528 (1991).
13. R. D. Fields, C. Yu, P. G. Nelson, *J. Neurosci.* **11**, 134 (1991).
14. We attached polytetrafluoroethylene (PTFE) inserts to culture dishes as described (*5*). The DRG neurons (50,000 per side compartment) from 13.5-day-old mouse fetuses were cultured in medium containing horse serum, NGF, and mitotic inhibitors to prevent Schwann cell proliferation (*13*). Three to four weeks after plating, the cultures were washed in medium lacking added NGF to eliminate the influence of NGF on L1 expression (*10*), and neurons were stimulated for 1 to 5 days with a

constant voltage stimulator as described (5). Total RNA (0.5 μg) was extracted [P. Chomcynski and N. Sacchi, *Anal. Biochem.* **162**, 156 (1987)], reverse transcribed by superscript ribonuclease H⁻ (Gibco BRL) with random primers, and amplified by PCR with primers corresponding to nucleotides 3476 through 3499 and 3611 through 3587 of the mouse L1 complementary DNA sequence of M. Moos *et al.* [*Nature* **334**, 701 (1989)] for 28 cycles at an annealing temperature of 57°C. The L1 fragment corresponded to the cytoplasmic domain and included the alternative splicing region characteristic of Schwann cell L1 [L. M. Moscoso and J. R. Sanes, *J. Comp. Neurol.* **352**, 321 (1995)]. This was done to detect possible Schwann cell contamination in these cultures (none was observed). The PCR products were analyzed on 6% polyacrylamide gels and quantified by imaging densitometry (Universal Imaging, West Chester, PA).

15. The competitive L1 mimic was constructed from a 225–base pair (bp) nonhomologous DNA fragment (Bam HI–Eco RI fragment of viral oncogene *v-erbB*) ligated to sequences complementary to the L1-specific primers used to amplify endogenous L1, according to the manufacturer's protocol (Clontech, Palo Alto, CA).
16. No difference was observed in total protein content, number of DRG neurons, mean diameter of soma, shape of the size-frequency histograms, or percentage of neurons staining for neurofilament (RT97) specific for large-type DRG neurons (about 75%) [S. N. Lawson, A. A. Harper, E. I. Harper, J. A. Garson, B. H. Anderton, *J. Comp. Neurol.* **228**, 263 (1984)] after 0, 0.1, or 1 Hz stimulation for 5 days.
17. We performed SDS–polyacrylamide gel electrophoresis as described (*10*) and quantified results by imaging densitometry, with polyclonal antibodies to L1 from mouse brain [F. G. Rathjen and M. Schachner, *EMBO J.* **3**, 1 (1984)] and NCAM antibody [E. Bock *et al.*, *ibid.* **4**, 2765 (1985)]. Schwann cells were not detected in these cultures, and the 230-kD band was distinct from the lower weight isoform of L1 expressed by Schwann cells (*6, 7*).
18. Axons were stained with antibody against neurofilament, and automated image analysis was used to quantify the number of axonal bundles intersecting a 315-μm linear transect in the side compartment, parallel to the partition and 100 to 200 μm from the barrier. Statistical comparisons by analysis of variance (ANOVA) were based on the mean number of fascicle intersections from 10 to 15 measurements in each dish (n = 26 dishes).
19. Schwann cells were prepared as described (*11*) and plated on DRG cultures (0.15×10^6 cells per dish) after 5 days of stimulation. Cultures were fixed with 4% paraformaldehyde 4 days later and stained with monoclonal antibody against the Schwann cell marker CNPase [T. J. Sprinkle *et al.*, *Brain Res.* **426**, 349 (1987)]. Quantitative comparisons were based on the mean number of Schwann cells per 0.13 mm^2 determined from at least 15 microscopic fields in each dish. Preincubation with antibody against L1 inhibited Schwann cell adhesion by >30% ($P < 0.009$; $n = 7$).
20. We are grateful to P. Nelson for continuous helpful discussions and to M. O'Donovan for reading an early draft of this report.

17 May 1995; accepted 18 September 1995

Scope of the AIDS Epidemic in the United States

Philip S. Rosenberg

Two-dimensional deconvolution techniques are used here to reconstruct age-specific human immunodeficiency virus (HIV) infection rates in the United States from surveillance data on acquired immunodeficiency syndrome (AIDS). This approach suggests that 630,000 to 897,000 adults and adolescents in the United States were living with HIV infection as of January 1993, including 107,000 to 150,000 women. The estimated incidence of HIV infection declined markedly over time among white males, especially those older than 30 years. In contrast, HIV incidence appears to have remained relatively constant among women and minorities. As of January 1993, prevalence was highest among young adults in their late twenties and thirties and among minorities. An estimated 3 percent of black men and 1 percent of black women in their thirties were living with HIV infection as of that date. If infection rates remain at these levels, HIV must be considered as endemic in the United States.

Through the use of deconvolution methods known as backcalculation, the national AIDS database compiled by the Centers for Disease Control and Prevention (CDC) and the distribution of the incubation period between infection with HIV and diagnosis with AIDS can be used to reconstruct the historical incidence of HIV infection that best accounts for the observed epidemic of AIDS cases (*1*). In recent years, the incidence of AIDS in the United States has slowed, with rates that are approaching a plateau (*2*). This trend likely reflects both reduced infection rates since the mid-1980s and widespread use of prophylactic therapies, which delay the onset of AIDS (*3*). Although the plateau in national AIDS cases would appear to be a favorable sign, optimism must be tempered by an appreciation of the dynamic nature of the epidemic. The HIV virus entered the United States during the late 1970s and spread rapidly during the early to mid-1980s. During this early period, there were large susceptible populations at risk over a broad range of ages. As the epidemic matured, one would expect that new entrants to at-risk populations—homosexual men, injection drug users, and high-risk heterosexuals—would tend to be young. Hence, it is plausible that the epidemic would stabilize with fewer infections occurring in recent years compared to the mid-1980s. For purposes of epidemic monitoring, therefore, a key question is whether the incidence rate among young adults has declined in comparison to the rate among persons of the same age in the past.

Although there are only limited data available to address this question by direct observation, extensions of the backcalculation approach (*4, 5*) allow one to estimate the age-specific incidence of infection from the age-specific incidence of AIDS. To avoid potential biases resulting from CDC's expansion of the AIDS case definition in 1993 (*2*), this approach was applied to cases among adults and adolescents who were diagnosed up to 1 January 1993 and who met the previous 1987 case definition (*6*). Incidence counts were adjusted for delays in reporting and estimates were inflated by 18% to reflect cases that will never be reported (*7*).

Although the overall rate of increase slowed after 1987, AIDS incidence trends differ according to birth cohort (Fig. 1A). Incidence among persons born before 1960 increased during the early to mid-1980s and then approached a plateau during 1991 to 1992, but AIDS incidence among persons born after 1960 was very low until 1986 and has increased steadily since then. This qualitative difference is apparent among men and women in each racial and ethnic group (Fig. 1B).

The infection rate function $\nu(s, a)$ specifies the number of infections per year at calendar time s among persons aged a years. Estimates of $\nu(s, a)$ were derived from age-specific AIDS incidence data on the basis of the incubation distribution. The fundamental convolution equation (*4, 5*) is given by

$$E(Y_{t,k}) = \int_{T_0}^{t} \nu(s,k-t+s) f(t-s \mid k-t+s,s)\,ds \quad (1)$$

where $E(Y_{t,k})$ is the expected number of cases occurring at calendar time t among persons aged k years at diagnosis, T_0 is the assumed start date of the epidemic, and $f(t \mid a, s)$ is the incubation period density function for persons aged a years at infection who were infected at time s. The incubation distribution varies by age to reflect that younger age is associated with slower progression (*8*) and by time to account for the increasing use of prophylactic therapies since 1987 (*1, 3–5*). Given observed AIDS incidence data $Y_{t,k}$ and an

National Cancer Institute, Rockville, MD 20852–4910, USA.