the molecular mechanisms underlying these adaptive responses at a cellular level. Long-term ethanol treatment may increase opiate binding in NG108-15 by increasing receptor synthesis, decreasing receptor degradation or processing, or increasing the availability of latent or hidden membrane receptors to bind opiate ligands (*17*).

The changes in receptor binding we describe may parallel the clinical phenomena of intoxication, tolerance, and withdrawal. For example, decreased binding produced by ethanol in the short-term could lead to depression of some receptor-mediated activity. During continued ethanol exposure the cell membrane may undergo adaptive changes, causing increased receptor density and restored cellular function. After abrupt ethanol withdrawal, the increased number of receptors would no longer be inhibited by ethanol, resulting in heightened receptor-mediated activity. With further passage of time, the number of receptors would return to control values, again restoring cellular homeostasis.

MICHAEL E. CHARNESS
Ernest Gallo Clinic and Research Center, Department of Neurology, University of California, San Francisco 94143
ADRIENNE S. GORDON
Ernest Gallo Clinic and Research Center, Departments of Pharmacology and *Neurology, University of California, San Francisco*
IVAN DIAMOND
Ernest Gallo Clinic and Research Center, Departments of Neurology, Pediatrics, and *Pharmacology, University of California, San Francisco*

References and Notes

1. J. H. Chin and D. B. Goldstein, *Science* **196**, 684 (1977); M. W. Hill and A. D. Bangham, *Adv. Exp. Med. Biol.* **59**, 1 (1975); P. Seeman, *Pharmacol. Rev.* **24**, 583 (1972); H. Rottenberg, A. Waring, E. Rubin, *Science* **213**, 583 (1981).
2. R. A. Harris and F. Schroeder, *Mol. Pharmacol.* **20**, 128 (1981); J. H. Chin and D. B. Goldstein, *ibid.* **13**, 435 (1977).
3. D. A. Johnson, N. M. Lee, R. Cooke, H. H. Loh, *ibid.* **15**, 739 (1979); R. C. Lyon and D. B. Goldstein, *ibid.* **23**, 86 (1983); L. M. Parsons, E. J. Gallaher, D. B. Goldstein, *J. Pharmacol. Exp. Ther.* **223**, 472 (1982); J. H. Chin, L. M. Parsons, D. B. Goldstein, *Biochim. Biophys. Acta* **513**, 358 (1978); A. J. Waring, H. Rottenberg, T. Ohnishi, E. Rubin, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2582 (1981); T. L. Smith and M. J. Gerhart, *Life Sci.* **31**, 1419 (1982); J. M. Littleton, G. R. John, S. J. Grieve, *Alcohol. Clin. Exp. Res.* **3**, 50 (1979).
4. L. P. Solomonson, V. A. Liepkalns, A. A. Spector, *Biochemistry* **15**, 892 (1976); B. H. Ginsberg, J. Jabour, A. A. Spector, *Biochim. Biophys. Acta* **690**, 157 (1982).
5. R. A. Rabin *et al.*, *J. Pharmacol. Exp. Ther.* **213**, 491 (1980); V. T. Tran, S. H. Snyder, L. F. Major, R. J. Hawley, *Ann. Neurol.* **9**, 289 (1981); R. A. Rabin and P. B. Molinoff, *J. Pharmacol. Exp. Ther.* **216**, 129 (1981).
6. S. Stenstrom and E. Richelson, *J. Pharmacol. Exp. Ther.* **221**, 334 (1982).
7. J. M. Hiller, L. M. Angel, E. J. Simon, *Science* **214**, 468 (1981).
8. A. Pfeiffer, B. R. Seizinger, A. Herz, *Neuropharmacology* **20**, 1229 (1982).
9. B. Tabakoff and P. Hoffman, *Life Sci.* **32**, 197 (1983); H. A. Jorgeson and K. Hole, *Eur. J. Pharmacol.* **75**, 223 (1981); A. S. Levine, S. Hess, J. E. Morley, *Alcohol. Clin. Exp. Res.* **7**, 83 (1983).
10. P. J. Syapin and E. P. Noble, in *Biochemistry and Pharmacology of Ethanol*, E. Majchrowicz and E. P. Noble, Eds. (Plenum, New York, 1979), p. 521.
11. R. Fantozzi, D. Mulliken-Kilpatrick, A. J. Blume, *Mol. Pharmacol.* **20**, 8 (1981); P. Y. Law, D. S. Hom, H. H. Loh, *ibid.* **23**, 26 (1983).
12. C. B. Pert and S. H. Snyder, *Science* **179**, 1011 (1973).
13. Cells were harvested in 137 m*M* NaCl, 5 m*M* KCl, 6 m*M* glucose, 59 m*M* sucrose, 5 m*M* Hepes buffer, 0.17 m*M* $Na_2PO_4 \cdot 7H_2O$, and 0.22 m*M* KH_2PO_4 (*p*H 7.6). Mean cell volume was determined by computer-fitting a Gaussian curve to the cell volume frequency distribution obtained on an Elzone monitor (Particle Data, Inc., Elmhurst, Ill.). The system was calibrated with a latex bead. Control cells day 6, 3588 μm^3 (standard deviation, 197; $N = 6$); ethanol cells (200 m*M* for 24 hours) day 6, 3655 μm^3 (standard deviation, 334, $N = 5$) ($P > 0.5$).
14. N. P. Franks and W. R. Lieb, *Nature (London)* **300**, 487 (1982).
15. R. C. Lyon, J. A. McComb, J. Schreurs, D. B. Goldstein, *J. Pharmacol. Exp. Ther.* **218**, 669 (1981).
16. J. D. Sinclair, M. Rusi, M. M. Airaksinen, H. L. Altschuler, in *Beta-Carbolines and Tetrahydroisoquinolines*, F. Bloom, H. Barchas, M. Sandler, E. Usdin, Eds. (Liss, New York, 1982), p. 365; K. Blum, M. G. Hamilton, J. E. Wallace, in *Alcohol and Opiates*, K. Blum, Ed. (Academic Press, New York, 1977), p. 203.
17. D. Heron, M. Israeli, M. Hershkovitz, D. Samuel, M. Shinitsky, *Eur. J. Pharmacol.* **72**, 361 (1981).
18. We thank P. Y. Law for his gift of the NG108-15 cells and L. Corash for his assistance with the cell volume measurements.

12 September 1983; accepted 30 September 1983

The Human Gene for the β Subunit of Nerve Growth Factor Is Located on the Proximal Short Arm of Chromosome 1

Abstract. *Fragments of the recently cloned human gene for the β subunit of nerve growth factor (β-NGF) were used as hybridization probes in analyzing two sets of rodent-human somatic cell hybrids for the presence of human β-NGF sequences. Results from the first set of hybrids assigned the human β-NGF gene to chromosome 1 and ruled out the presence of sequences of comparable homology on any other chromosome. With the second set of hybrids, which contained seven different, but overlapping, regions of chromosome 1, the NGF locus was mapped to band 1p22.*

Nerve growth factor (NGF) is important in the development and function of peripheral adrenergic and sensory neurons and of adrenal chromaffin cells (*1*). As a polypeptide that binds to a specific receptor, NGF may serve as a model system for the action of hormones and growth factors. Furthermore, NGF appears to play a role in several human genetic disorders of the nervous system (*2*). In the mouse, the 118-amino-acid β chain, which is responsible for stimulating nerve growth, is synthesized as part of a larger precursor molecule (*3*). Oligonucleotide primers based on the known amino acid sequence of the gene (*4*) were synthesized and used to clone the mouse β-NGF gene (*5, 6*). Mouse β-NGF complementary DNA was then used to isolate the human β-NGF gene. Sequencing studies revealed considerable homology between human and mouse amino acid sequences (*6*). The β-NGF gene has not yet been assigned to a specific chromosome in any mammalian species.

The mapping of the human β-NGF locus is of particular interest for three reasons. First, β-NGF represents the active subunit of the NGF complex, which consists of three different polypeptide chains (α, β, and γ). The expression of these presumably independent genes appears to be synchronized during development and adulthood. Identification of the chromosomal locations of these genes will help to determine whether synchronized gene expression of the NGF subunit genes is due to regulation of one genetic locus containing a cluster of NGF subunit genes, or whether characteristics common to three physically separated genes control their synchronous expression.

Second, low levels of amino acid homology with insulin have suggested that β-NGF may be a member of the insulin gene family (*1*). The insulin locus is now well established on the distal short arm of human chromosome 11 (*7*), but other insulin-related genes have not yet been mapped. Knowledge about the chromosomal location of each member of a gene family can contribute to our understanding of the patterns and mechanisms of genome evolution (*8*). In this context, the insulin gene family represents an interesting case, in that its members (IGF-1, IGF-2, relaxin, insulin, and possibly β-NGF) have acquired different regulatory functions while retaining various amounts of structural homology.

Third, at least ten cellular proto-oncogenes (that is, sequences that are related to retroviral transforming genes or that have been identified in the activated state by transfection assays with human tumor cell DNA) have been assigned to specific human chromosomes or chromosome regions (*9*). A search for relations between proto-oncogenes and

known growth regulatory molecules could benefit from comparing the chromosomal locations of the two kinds of genes. No primary assignment of a growth-factor locus has as yet been made in the human. The gene for platelet-derived growth factor was assigned to chromosome 22 when its identity with the c-*sis* proto-oncogene was discovered (*10*).

For mapping of the human β-NGF locus we used two sets of previously established rodent-human somatic cell hybrids (*11*). The 15 hybrids in the first set were derived from two different Chinese hamster cell lines and six different human donors. They were selected for stable contents of overlapping sets of human chromosomes (Table 1). Southern blots prepared with Eco RI–digested DNA from hybrid cell lines and from human and rodent controls were probed with ^{32}P-labeled human NGF fragments (Fig. 1C) (*12*). Human DNA samples revealed two bands of hybridization, 6.7 kilobases (kb) and 1.0 kb in size (lane 8 in Fig. 1A). The Chinese hamster parental cell line V79/380-6 produced two fragments of approximately 8 kb and 3.7 kb (lane 7 in Fig. 1A). Similar fragments were detected in all Chinese hamster

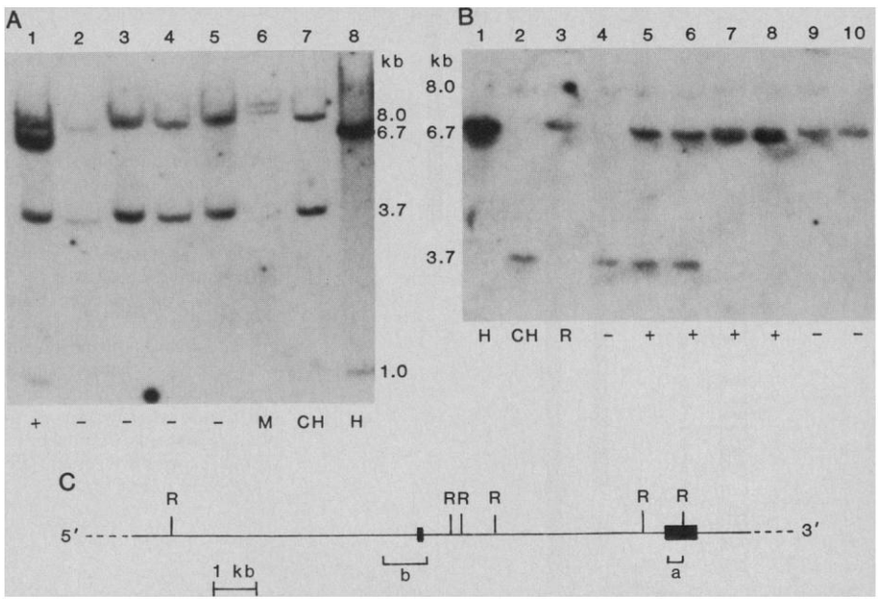


Fig. 1. (A and B) Autoradiograms of nitrocellulose filters carrying Eco RI–cleaved DNA from rodent parental cell lines: mouse 3T3TK$^-$ (*M*), Chinese hamster V79/380-6 (*CH*), and rat hepatoma 7777 (*R*), from human fibroblasts (*H*) and from rodent-human somatic cell hybrids. Lanes 1 to 5 in (A) and lanes 4 to 6 in (B) are Chinese hamster–human hybrids; lanes 7 to 10 in (B) are rat-human hybrids. Filters were hybridized with ^{32}P-labeled (*21*) probes a and b derived from subcloned fragments of recombinant λ clones (*6*). Probe a was a 310-base-pair Eco RI–Pst I-fragment. Locations of these fragments are indicated on the physical map. (C) Boxes indicate exons of the human β-NGF gene. *R* stands for Eco RI sites. Hybridization was carried out overnight at 42°C in the presence of 50 percent formamide. Filters were washed three times in 0.2× standard saline citrate at 42°C. Hybrids were scored as positive (+) or negative (−) for the human 6.7-kb and 1.0 fragments (A) or for the 6.7-kb fragment only (B).

Table 1. Assignment of the β-NGF locus to chromosome 1. The presence (+) or absence (−) of human chromosomes was determined by karyotype analysis of 15 to 30 cells, with + indicating the presence of at least one copy of the chromosome in 10 percent of the cells (frequency of 0.1). For chromosome 1 the frequencies are specified. The presence of human restriction fragments containing NGF sequences was compared with the chromosome content of these hybrids, and for each chromosome the ratio of discordant hybrids to the total number of informative hybrids was calculated. Data on chromosomes that were only present in part (P) because of rearrangements were excluded from this analysis. Concordancy was observed only for chromosome 1 with all other chromosomes excluded by four or more discordant hybrids.

Hybrid clones	Human chromosomes																							
	β-NGF	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
XII-2D	+	.6	+	+	+	-	+	-	+	+	-	-	+	+	+	+	+	-	+	+	-	+	+	P	-
XII-2D aza	-	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-	+	-	-	-
XII-12B aza	+	.9	-	+	+	-	+	-	+	-	-	-	+	-	+	+	+	-	+	+	-	+	+	P	-
XIII-3C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	+	-	-
XIII-4C aza	+	.1	-	-	-	-	+	-	+	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-	-
XIII-4D-1c aza	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
XV-G1	-	-	+	P	+	+	-	-	+	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	-
XV-F4	-	P	+	+	-	+	+	-	+	-	-	-	-	+	+	-	+	-	+	+	+	+	+	-	-
XVII-10A-12a	-	-	-	+	P	-	+	+	-	-	+	+	+	-	-	+	-	-	-	-	+	+	+	+	-
XVII-18B-10a	-	-	+	+	-	-	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	+	-	+	-
XVIII-54A aza	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	-	+	-	+	-	+	-	-	+	-
XXI-22A-g-1a	-	-	-	-	-	+	+	-	+	-	-	+	-	+	-	+	-	+	+	-	+	+	+	-	-
XXI-22A-h	+	.3	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	-	-	+	+	+	-
XXI-23A-2c	+	.9	-	-	-	-	-	-	+	-	-	P	-	-	+	-	-	+	-	-	-	-	+	+	-
XXI-42A	-	-	-	+	-	+	-	-	+	-	+	+	+	-	+	-	-	+	-	-	-	+	+	+	-
Ratio of discordant to total hybrids		0/14	8/15	9/14	4/14	9/15	8/15	5/15	4/15	4/15	8/15	8/14	7/15	8/15	7/15	5/15	8/15	6/15	4/15	5/15	10/15	8/15	7/15	6/13	6/15

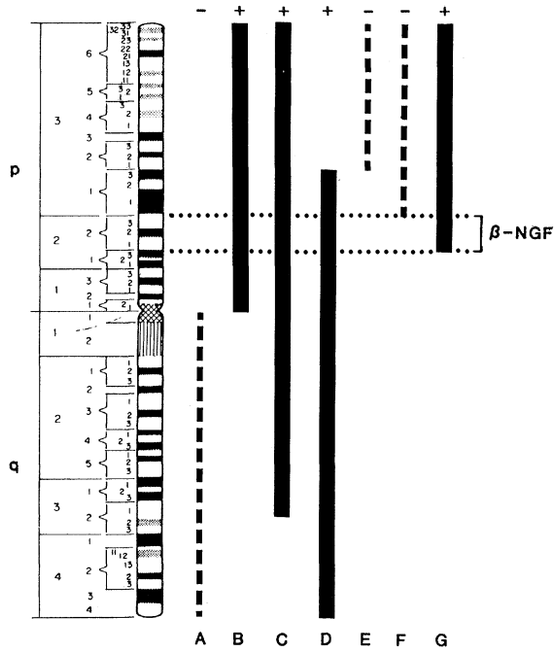


Fig. 2. Idiogram of trypsin-Giemsa banding patterns of human chromosome 1 (*22*). Bars A to G represent regions retained in the different types of hybrids. Presence or absence of the human 6.7-kb Eco RI fragment is indicated by + or −. As judged from these results, the NGF locus is excluded from regions represented by hatched bars. Its most likely location is in the shortest region of overlap of all positive hybrids (region p31.1 to p22.1, which is essentially all of band p22).

hybrids whether the parental line was derived from V79 (lanes 2 to 4 in Fig. 1A) or from Don (lanes 1 and 5 in Fig. 1A). Both human fragments were present in positive hybrids such as the one shown in lane 1 in Fig. 1A. In some autoradiograms, the larger fragment was more easily detected than the 1.0-kb fragment. Therefore, the hybrids were scored primarily for the presence of the 6.7-kb fragment. Concordant segregation of this fragment was observed only with chromosome 1. All other chromosomes were excluded by four or more discordant hybrids (Table 1).

Since human chromosome 1 contains almost 10 percent of the total autosomal genetic information, regional mapping of the β-NGF locus was desirable. The ten hybrids in the second set were derived from Chinese hamster, mouse, or rat cell lines fused to one of four different human donor cell strains. These hybrids contained seven different well-defined and partially overlapping regions of chromosome 1 in the absence of a normal chromosome 1 (*13*). Filters carrying Eco RI–cleaved DNA's of these hybrids and of the parental cell lines were incubated with human NGF probe under stringent conditions of hybridization (Fig. 1B). DNA from the rat parental cell line produced a major fragment of 6.9 kb, only slightly larger than the human 6.7-kb fragment (lane 3 in Fig. 1B). However, it was possible to score for the human fragment in rat-human somatic cell hybrids, two of which displayed the human fragment (lanes 7 and 8 in Fig. 1B), whereas two others displayed only the rat fragment (lanes 9 and 10 in Fig. 1B). Hybrids in lanes 9 and 10 contained the long arm of chromosome 1 and those in lanes 7 and 8 contained only the short arm. Of the Chinese hamster–human hybrids, the one in lane 6 contained region C of chromosome 1 (Fig. 2), and the one in lane 5 contained region D. Both of them were positive for the human fragment, whereas the hybrid in lane 4 that contained region E (the part missing from the hybrid in lane 5), did not have the human 6.7-kb fragment. Of the two mouse-human hybrids that contained either region F or G of chromosome 1, only the one with region G had human NGF sequences (data not shown). Figure 2 summarizes the regional mapping data and illustrates the region of the short arm of chromosome 1 that contains the human β-NGF locus.

Our results indicate the existence of a single locus for β-NGF, mapped to the same chromosome region to which we have previously assigned the N-*ras* proto-oncogene (*14*). More recent studies have revealed that N-*ras* maps to the same band as β-NGF (1p22) (*14*). This oncogene has been identified as the transforming sequence in the HL-60 human promyelocytic leukemia cell line and also in the SK-N-SH neuroblastoma cell line (*15*). It is distantly related to the *ras* family of viral and cellular oncogenes (*15*). Whether there is any relation between β-NGF and N-*ras* remains to be investigated. The localization of β-NGF on band 1p22 places this gene in measurable distance from other polymorphic markers in that region. Thus, family linkage studies of restriction fragment–length polymorphisms detected with the β-NGF probe (*16*) could reveal linkage to the amylase loci, Charcot-Marie-Tooth neuropathy, the Duffy blood group, and phosphoglucomutase 1, as well as to heteromorphisms of the 1qh region (*17*). Furthermore, breaks in the short arm of chromosome 1, often leading to partial deletions, are the most consistent structural chromosome abnormality reported in human neuroblastomas (*18*). Studies of some human neuroblastomas in vitro have shown that maturation of neuroblasts to ganglion cells occurs under the influence of NGF (*19*). Therefore, deletion of chromosome arm 1p—if it includes the β-NGF locus—in a neuroblast during early embryonic development could cause delay of normal differention and thus predispose the blast cell to malignant transformation. Such a mechanism would be analogous to deletions of band 13q14 associated with retinoblastoma and deletion of band 11p13 leading to predisposition to nephroblastoma [reviewed in (*20*)]. Studies of β-NGF gene copy number and organization in human neuroblastomas will be necessary to evaluate this hypothesis.

UTA FRANCKE
BÉRENGÈRE DE MARTINVILLE
Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut 06510
LISA COUSSENS
AXEL ULLRICH
Department of Molecular Biology, Genentech Inc., South San Francisco, California 94080

References and Notes

1. R. A. Bradshaw, *Annu. Rev. Biochem.* **47,** 191 (1978); G. P. Harper and H. Thoenen, *Annu. Rev. Pharmacol. Toxicol.* **21,** 205 (1981); B. A. Yankner and E. M. Shooter, *Annu. Rev. Biochem.* **51,** 845 (1982); R. Levi-Montalcini, *Annu. Rev. Neurosci.* **5,** 341 (1982); J. Pearson, E. M. Johnson, L. Brandeis, *Dev. Biol.* **96,** 32 (1983).
2. W. C. Mobley, A. C. Server, D. N. Ishii, R. J. Riopelle, E. M. Shooter, *N. Engl. J. Med.* **297,** 1211 (1977); J. P. Schwartz and X. O. Breakefield, *Proc. Natl. Acad. Sci. U.S.A.* **77,** 1154 (1980); R. N. Fabricant and G. J. Todaro, *Arch. Neurol.* **38,** 401 (1981); K. DeSchryver-Kecskemeti, R. E. Clouse, M. N. Goldstein, D. Gersell, L. O'Neal, *N. Engl. J. Med.* **308,** 635 (1983).
3. E. A. Berger and E. M. Shooter, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 3647 (1977).
4. R. H. Angeletti and R. A. Bradshaw, *ibid.* **68,** 2417 (1971).
5. J. Scott, M. Selby, M. Urdea, M. Quiroga, G. I. Bell, W. J. Rutter, *Nature (London)* **302,** 538 (1983).
6. A. Ullrich, A. Gray, C. Berman, T. J. Dull, *ibid.* **303,** 821 (1983).
7. M. E. Harper, A. Ullrich, G. F. Saunders, *Proc. Natl. Acad. Sci. U.S.A.* **78,** 4458 (1981); U. Francke and B. de Martinville, in *Recombinant DNA Applications to Human Disease,* T. Caskey and R. White, Eds. (Banbury Report 14, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1983), p. 175; B. de Martinville and U. Francke, *Nature (London)* **305,** 641 (1983).
8. T. Ohta and G. A. Dover, *Proc. Natl. Acad. Sci. U.S.A.* **80,** 4079 (1983).
9. D. C. Swan, O. W. McBride, K. C. Robbins, D. A. Keithley, E. P. Reddy, S. A. Aaronson, *ibid.* **79,** 4691 (1982); R. Dalla-Favera, R. C. Gallo, A. Giallongo, C. M. Croce, *Science* **218,** 686 (1982); R. Dalla-Favera, G. Franchini, S. Martinotti, F. Wong-Stahl, R. C. Gallo, C. M. Croce, *Proc.*

Natl. Acad. Sci U.S.A. **79,** 4714 (1982); B. G. Neel, S. C. Jhanwar, R. S. K. Chaganti, W. S. Hayward, *ibid.*, p. 7842; N. Heisterkamp *et al.*, *Nature (London)* **299,** 747 (1982); O. W. McBride, D. C. Swan, E. Santos, M. Barbacid, W. R. Tronick, S. A. Aaronson, *ibid.* **300,** 773 (1982); B. de Martinville, J. Giacalone, C. Shih, R. A. Weinberg, U. Francke, *Science* **219,** 498 (1983); A. Y. Sakaguchi, S. L. Naylor, T. B. Shows, J. J. Toole, M. McCoy, R. A. Weinberg, *ibid.*, p. 1081.
10. R. F. Doolittle *et al.*, *Science* **221,** 275 (1983); M. D. Waterfield *et al.*, *Nature (London)* **304,** 35 (1983); C. D. Stiles, *Cell* **33,** 653 (1983).
11. U. Francke, N. Busby, D. Shaw, S. Hansen, M. G. Brown, *Somat. Cell Genet.* **2,** 27 (1976); U. Francke and B. Francke, *ibid.* **7,** 171 (1981); see also (*13*).
12. E. Southern, *J. Mol. Biol.* **98,** 503 (1975).
13. Hybrids with regions C, D, and E (Fig. 2) were derived from human donors with balanced translocations of chromosome 1 [U. Francke, *Cytogenet. Cell Genet.* **14,** 308 (1975); _____ and M. A. Pellegrino, *Proc. Natl. Acad. Sci. U.S.A.* **74,** 1147 (1978); *ibid.*, p. 5776]. Hybrids with regions A, B, F, and G had acquired the rearrangements of chromosome 1 during evolution in vitro of the hybrid cell lines [D. L. George and U. Francke, *Hum. Hered.* **28,** 161 (1978); S. Vora, S. Durham, B. de Martinville, D. L. George, U. Francke, *Somat. Cell Genet.* **8,** 95 (1982)].
14. B. de Martinville, J. M. Cunningham, M. J. Murray, U. Francke, *Nucleic Acids Res.* **11,** 5267 (1983); B. de Martinville, M. Muenke, J. M. Cunningham, M. J. Murray, U. Francke, *Cytogenet. Cell Genet.*, in press.
15. M. J. Murray, J. M. Cunningham, L. F. Parada, F. Dautry, P. Lebowitz, R. A. Weinberg, *Cell* **33,** 749 (1983); K. Shimizu, M. Goldfarb, M. Perucho, M. Wigler, *Proc. Natl. Acad. Sci. U.S.A.* **80,** 383 (1983).
16. X. O. Breakefield, G. Orloff, C. M. Castiglione, in *Biochemical and Clinical Aspects of Neuropeptides: Synthesis, Processing and Gene Structure.* G. Koch and D. Richter, Eds. (Academic Press, New York, in press).
17. "Human Gene Mapping 6," *Cytogenet. Cell Genet.* **32,** 341 (1982).
18. V. B. Babapulle and N. B. Atkin, *Cancer Genet. Cytogenet.* **4,** 215 (1981); M. M. Haag, S. W. Soukup, J. E. Neely, *Cancer Res.* **41,** 2995 (1981); F. Gilbert, G. Balaban, P. Moorhead, D. Bianchi, H. Schlesinger, *Cancer Genet. Cytogenet.* **7,** 33 (1982); G. M. Brodeur, A. A. Green, F. A. Hayes, K. J. Williams, D. L. Williams, A. A. Tsiatis, *Cancer Res.* **41,** 4678 (1981).
19. T. Waris, L. Rechardt, P. Waris, *Experientia* **29,** 1128 (1973); A. R. Kolber, M. N. Goldstein, B. W. Moore, *Proc. Natl. Acad. Sci. U.S.A.* **71,** 4203 (1974); C. P. Reynolds and J. R. Perez-Polo, *Neurosci. Lett.* **1,** 91 (1975).
20. U. Francke, in *Chromosomes and Cancer: From Molecules to Man*, J. D. Rowley and J. E. Ultmann, Eds. (Academic Press New York 1983), p. 99.
21. J. M. Taylor, R. Illmensee, J. Summers, *Nucleic Acids Res.* **9,** 2313 (1981).
22. U. Francke, *Cytogenet. Cell Genet.* **31,** 24 (1981).
23. Supported by NIH research grant GM 26105 (to U.F.) and Genentech Inc.
* Address correspondence to U.F.

31 August 1983; accepted 6 October 1983

Time Course of α-Flupenthixol Action Explains "Response Artifacts" of Neuroleptic Action on Brain Stimulation Reward

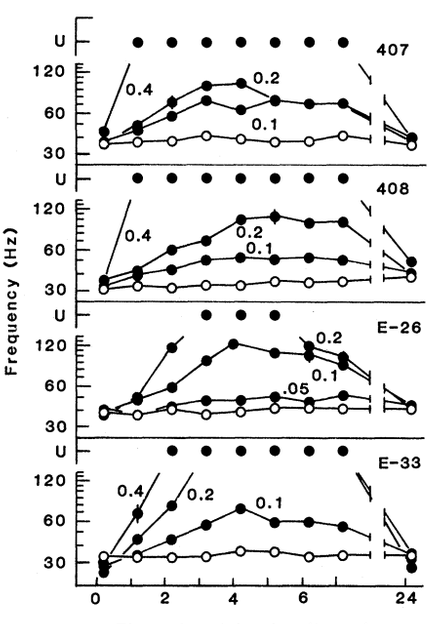
Ettenberg *et al.* (*1*) reported that doses of the neuroleptic α-flupenthixol that spared nose poking for lateral hypothalamic self-stimulation markedly suppressed bar pressing for the same reward. Central to their interpretations of this apparently task-dependent drug effect was the notion that the task used to earn brain stimulation somehow modulates the degree to which dopamine cells participate in reward.

We do not consider the viability of their interpretations because the experiment itself suffers a serious design error. The drug effect on nose poking was assessed 2.5 hours after injection, and bar-pressing tests were held about 50 minutes afterward. The task order was not counterbalanced: "The effects of each dose were tested on nose poking for brain stimulation and then on lever pressing" (*1*, p. 358). If these tests were held over the rising phase of receptor concentration, then for any particular dose the effective antagonism would be consistently lower in the nose-poke trials than in the bar-press tests. It thus would come as no surprise that nose-poking behavior survived at a dose that completely eliminated bar pressing.

To see whether α-flupenthixol's behavioral effect attains asymptote at 2 or even 3 hours after injection, we ran a time-course study of its action on lateral hypothalamic self-stimulation. Required frequencies (*2*) needed to sustain criterion bar-press rates were determined at hourly intervals beginning immediately after injection. This measure is equivalent to "threshold" determination; the sole difference is that reward summation functions (*3*) are cut at moderate levels of performance instead of at just noticeable departures from zero responding. Often, high doses suppressed responding altogether. Required frequencies could not be measured and these undetermined points are shown as unconnected dots against "U" of Fig. 1. Lower doses caused required frequencies to climb, on average, through to the 4-hour test. The implication is that Ettenberg *et al.* conducted their tests too soon after drug administration; by failing to counterbalance task order, they effectively assessed the two tasks with different pharmacological populations.

We then attempted to replicate their result with tests that (i) began after a longer postinjection interval (3 hours, 45 minutes) and (ii) included both orders of task presentation. Each behavior was tested daily for 20 minutes and the two sessions were separated by 20 minutes. Doses of α-flupenthixol were given every other day (*4*). The dose of 0.4 mg/kg completely abolished bar pressing and nose poking (Fig. 2), a result that is at odds with the spared nose poking reported at this dose and twice this dose by Ettenberg *et al.* Our failure to replicate fits with a report (*5*) that haloperidol reduces performance of these two operants to the same degree. Bar-pressing performance was more reduced than nose poking at our lower doses; we agree with Ettenberg *et al.* that this latter result cannot be taken as a task-dependent difference in substrate sensitivity.

Our first experiment demonstrates



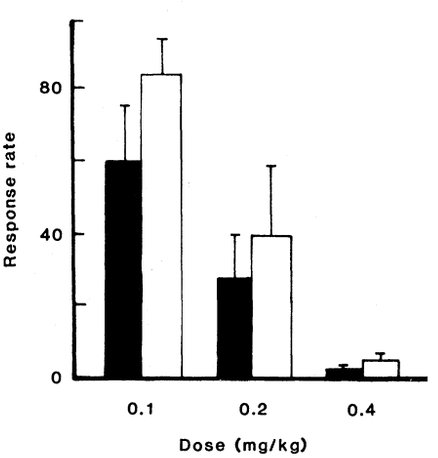


Fig. 1 (left). Time-course data. Frequency of stimulation pulses required to obtain criterion responding after administration of α-flupenthixol. Open circles represent saline tests and closed circles depict drug tests. Doses, in milligrams per kilogram, are shown beside the curves. Each symbol indicates the geometric mean and standard error of four measurements. Points at the "U" level are undetermined. Fig. 2 (right). Effects of α-flupenthixol on lever pressing (closed bar) and nose poking (open bar) for lateral hypothalamic stimulation. The mean response rate is expressed as a percentage of the saline control. These data illustrate that α-flupenthixol has equally disruptive effects on both tasks.