by administration in vivo. That high doses of erythrosine affected behavior in a punishment paradigm indicates that further studies on the biological actions of this compound are necessary. Although hyperkinesis is a medical problem, the suggestion that it may be due to synthetic food additives has given it social and political dimensions that increase the need for sound clinical and basic data upon which to make policy judgments. Whatever the outcome of future scientific and clinical experimentation, cautious presentation and interpretation of data will prevent expensive and spurious perturbations of the public and scientific consciousness.

Note added in proof: Locomotor activity was also measured in adult (220 to 290 g) male rats. Activity before drug was 728 ± 65 counts per 60 minutes. Rats (eight in each group) were given erythrosine (100 mg/kg; 50 mg/ml) or saline intraperitoneally. The results were, for the erythrosine-treated rats, 870 ± 146 counts per 120 minutes, and for the saline-treated rats, 326 ± 81 counts per 120 minutes; $.05 < P < .1$, two-tailed t-test.

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Compensatory Increase in Tyrosine Hydroxylase Activity in Rat Brain After Intraventricular Injections of 6-Hydroxydopamine

Abstract. *The neurotoxin 6-hydroxydopamine produced a permanent loss of endogenous norepinephrine and of ^{3}H-labeled norepinephrine uptake sites in the hippocampus within 5 days. These losses were initially accompanied by parallel decreases in tyrosine hydroxylase activity and synaptosomal norepinephrine synthesis. Within 21 days, however, hippocampal tyrosine hydroxylase activity and norepinephrine synthesis rate increased three- to fivefold. These data suggest a novel form of plasticity in brain-damaged animals characterized by an increase in the capacity for transmitter biosynthesis in residual neurons.*

Systemic administration of 6-hydroxydopamine (6-OHDA) destroys most noradrenergic nerve endings in the sympathetic nervous system of the rat. However, the catecholamine-containing chromaffin cells of the adrenal medulla are not destroyed, and within 2 days, the activity of tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, is increased in these cells (*1*, *2*). We now report that an analogous process occurs in the central nervous system. The loss of central noradrenergic terminals following the administration of intracerebroventricular 6-OHDA leads to a rapid increase in TH activity in neuronal cell bodies, followed by an increase in TH activity and in norepinephrine (NE) synthesis in residual terminals. These results are discussed in terms of the ability of rats to sustain extensive damage to central catecholaminergic systems with relatively little functional impairment.

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Adult male Sprague-Dawley rats (Zivic-Miller) weighing 200 to 250 g on arrival were housed individually in hanging wire cages illuminated from 0700 to 1900 hours and kept at temperatures between 23° and 25°C. They were allowed free access to Purina rat chow and tap water. After 3 to 5 days, rats were anesthetized with ether and given a single injection of 6-OHDA (250 μg of free base in 20 μl of vehicle) or vehicle (0.9 percent NaCl, 0.1 percent ascorbic acid) into the lateral ventricle (*3*). Animals were killed by decapitation 5, 21, or 76 days later, and their brains were rapidly removed and placed on ice. The locus coeruleus region (average weight, 2 mg), containing noradrenergic cell bodies, was circumscribed bilaterally and dissected out (*4*). The hippocampus (average weight, 90 mg), selected to represent a noradrenergic terminal field, was also removed bilaterally.

Soluble TH activity was measured by a modification of previous methods (*5*). Tissue stored at −70°C for as long as several weeks was homogenized in 50 m*M* tris-HCl buffer (*p*H 6.0) and centrifuged at 49,500*g* for 1 hour. Supernatant was then incubated for 10 to 50 minutes at 37°C in 0.12*M* tris-acetate buffer (*p*H 6.2) in the presence of 75 μM L-[1-^{14}C]tyrosine (50 mCi/mmole, New England Nuclear), 3 m*M* 6-methyl-5,6,7,8-tetrahydropterin HCl ($6MPH_4$, Calbiochem), catalase, dihydropteridine reductase, and the reduced form of nicotinamide adenine dinucleotide (NADH). The resulting L-[1-^{14}C]dihydroxyphenylalanine was subsequently decarboxylated by an excess of L-aromatic acid decarboxylase in the presence of 0.1*M* tris-acetate buffer (*p*H 7.3), pyridoxal-5′-phosphate, and 3-iodo-L-tyrosine. The $^{14}CO_2$ was trapped in NCS tissue solubilizer (Amersham) and counted. Norepinephrine was measured by a radiometric method in involving the methylation of NE by catechol-*O*-methyltransferase with radiolabeled *S*-adenosyl methionine as a methyl donor (*6*); and soluble protein content was determined by the method of Lowry *et al.* (*7*), with bovine serum albumin (BSA) as standard.

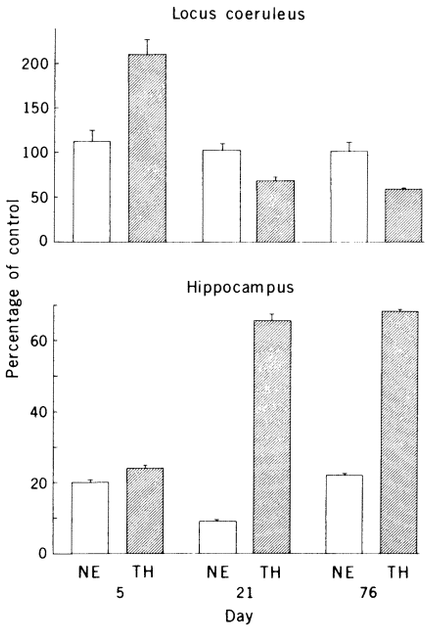

Fig. 1. Effect of 6-OHDA on NE content and TH activity in the locus coeruleus and the hippocampus. All values are expressed as mean percentage of the control (± S.E.) for at least seven samples. Mean control values: NE, 47 μg per pair (locus coeruleus) and 400 ng/g (hippocampus); TH activity, 5.5 pmoles per pair per minute (locus coeruleus) and 4.7 pmoles per milligram of protein per minute (hippocampus). All values were at least three times above the blank.

The 6-OHDA produced a permanent depletion of hippocampal NE to 20 percent of the control concentration with little effect on NE in the locus coeruleus. These findings are consistent with the hypothesis that 6-OHDA administered into the cerebrospinal fluid destroys noradrenergic terminals while leaving cell bodies intact (*8*). Five days after 6-OHDA administration, TH activity in the hippocampus was reduced to 24 percent of control. By 21 days, however, hippocampal TH activity had increased to 68 percent of control, where it remained on day 76. This increase in TH activity was preceded by a transient rise in enzyme activity in the locus coeruleus (Fig. 1) (*9*).

We sought to determine whether the enzyme activity measured in tissue from the animals with lesions resembled TH activity from control animals. A number of properties were examined, including the enzyme's requirement for O_2 and the cofactor, $6MPH_4$, its *p*H optimum, the effect of the TH inhibitor, 3-iodo-tyrosine, and its Michaelis constant (K_m) for cofactor and substrate. In each case, the behavior of samples from animals lesioned 21 days earlier paralleled that of control samples (Table 1). Thus, it appears that the same enzyme was being assayed in both groups of animals and that the changes observed cannot be attributed to a change in the affinity of the enzyme for either tyrosine or $6MPH_4$.

One possible explanation for the increase in TH activity after 6-OHDA administration was that collateral sprouting occurred from the sympathetic nerve terminals normally present within the hippocampus (*10*). To examine this hypothesis, the superior cervical ganglion, which contains the cell bodies of origin for these terminals (*11*), was removed unilaterally under ether anesthetic; the success of the operation was confirmed by the presence of eyelid ptosis. After 10 days, the rats were given 6-OHDA or vehicle as before. The animals were killed 21 days later. Ganglionectomy did not appear to alter TH activity in the ipsilateral hippocampus of vehicle-treated animals (mean activity: 2.87 pmole per milligram of protein per minute, ipsilateral; 2.08 pmole, contralateral control; N = 3). Moreover, the effect of 6-OHDA on TH activity in the ipsilateral hippocampus was identical to that in the contralateral side and to that previously observed in animals without ganglionectomies (75 percent of control, N = 3).

Table 1. Characteristics of hippocampal TH in control animals and those treated with 6-OHDA. The characteristics of hippocampal TH activity were determined 21 days following 6-OHDA treatment (250 μg, intraventricular). The *p*H values refer to the final *p*H for the TH reaction mixture. Anaerobic conditions consisted of flushing samples with N_2 for 1 minute before initiating the tyrosine hydroxylation reaction. Kinetic analysis of the enzyme from control and 6-OHDA animals indicated no change in the apparent K_m for either tyrosine (150 μM, control; 157 μM, 6-OHDA) or $6MPH_4$ (0.48 m*M*, control; 0.47 m*M*, 6-OHDA).

Incubation conditions	Tyrosine hydroxylase activity			
	Control		6-OHDA	
	Counts per minute	Percentage of standard conditions	Counts per minute	Percentage of standard conditions
Standard conditions	2043	100	1408	100
No $6MPH_4$	129	6.3	131	9.3
Anaerobic conditions	289	14.1	252	17.9
1.0 m*M* 3-iodo-L-tyrosine before TH reaction	209	10.2	168	11.9
Boiled tissue	229	11.2	190	13.5
No tissue	122	6.0	112	7.9
*p*H 5.7	1747	85.5	1129	80.2
*p*H 5.9	1923	94.1	1319	93.7
*p*H 6.5	1097	53.7	725	51.5

Table 2. Effect of 6-OHDA on NE uptake and synthesis in hippocampal synaptosomes. Values are expressed as mean (± standard error) and percentage of control values. The number of comparisons is indicated in parentheses.

Days after 6-OHDA injection	[^{3}H]NE uptake (nCi mg^{-1} min^{-1})			[^{14}C]NE synthesis (pmole mg^{-1} min^{-1})		
	Control	6-OHDA	Percentage of control	Control	6-OHDA*	Percentage of control
5 to 8	10.93 ± 0.50	1.45 ± 0.20	12.3 ± 2 (4)	1.00 ± 0.07	0.13 ± 0.008	13.3 ± 0.8 (6)
12 to 14				1.01 ± 0.07	0.30 ± 0.02	29.6 ± 2.2 (8)
21 to 22	10.00 ± 0.14	0.94 ± 0.05	9.4 ± 0.5 (4)	0.98 ± 0.05	0.66 ± 0.03	67.1 ± 1.1 (4)

*Increased significantly with time, as determined by analysis of variance ($P < .01$).

A second possibility for the rise in TH activity was that collateral sprouting or regeneration from central noradrenergic axons had taken place. To examine this possibility, we measured high-affinity uptake of ^{3}H-labeled NE into synaptosomes prepared from hippocampus. This uptake, which is characteristic of noradrenergic terminals, disappears after they are destroyed (*12*). Freshly dissected hippocampus was homogenized in 0.32*M* sucrose and centrifuged at 1100*g* for 10 minutes. The resulting supernatant was again centrifuged at 14,500*g* for 20 minutes and the pellet was used as a crude source of synaptosomes (*13*). The pellet was resuspended in 0.32*M* sucrose, and a portion was taken to determine NE and protein content. A second portion was incubated at 37°C in Krebs-Ringer phosphate buffer (*p*H 7.4) containing 2 μM DL-[7-^{3}H(*N*)]norepinephrine. After 5 minutes, samples were poured through a 45-μm filter (Millipore) moistened with the incubation medium containing 1 percent BSA and 0.5 m*M* NE. The filter was washed with the same medium (without BSA) at 37°C and placed in a scintillation vial to which was added 1 ml of 0.2*N* perchloric acid and then 10 ml of Scintiverse (Fisher Scientific). We observed that 5 days after 6-OHDA administration, the net accumulation of tritium was reduced in proportion to the loss of NE. These changes were still present on day 21 (Table 2). Collectively, these results suggest that the rise in hippocampal TH, which occurred by day 21, cannot be accounted for on the basis of the development of additional terminals from either peripheral or central noradrenergic axons.

In the last experiment, we determined whether the increase in TH activity between days 5 and 21 resulted in an increase in the ability of hippocampal tissue to synthesize NE. A crude synaptosomal fraction was prepared as before and incubated for 30 minutes at 37°C in Krebs-Ringer phosphate buffer (*p*H 7.4) in the presence of 8 μM L-[1-^{14}C]-tyrosine. The $^{14}CO_2$ was trapped and counted (*14*). Between 5 and 8 days after the 6-OHDA administration, in vitro catecholamine biosynthesis paralleled ^{3}H-labeled NE uptake. However, in contrast to uptake, synthesis increased fivefold over the next 2 weeks (Table 2).

We interpret these observations to indicate that 6-OHDA causes an initial loss of TH activity due to terminal degeneration, followed by a temporary increase in TH activity in noradrenergic cell bodies, and then by the gradual appearance of increased TH activity in residual nerve endings. The apparently long-lasting increase in TH activity in noradrenergic terminals is accompanied by an increase in the ability of terminals to synthesize catecholamines. The failure of catecholamine stores to increase despite the increase in biosynthetic capacity, also observed in the adrenal medulla after systemic 6-OHDA (*2*), may indicate that in vitro catecholamine turnover had increased as well. To our knowledge, such a sequence of events has not been described previously, although comparable but transient changes in TH activity in central noradrenergic cell bodies and terminal regions have been noted after the systemic administration of reserpine (*4, 15*). Moreover, in the striatum, both TH activity and dihydroxyphenylacetic acid concentration are less affected than is dopamine concentration after the intraventricular administration of 6-OHDA (*16, 17*).

The cause of the relative increase in TH activity and in vitro NE synthesis in the hippocampus remains to be determined. Two explanations appear to be the most likely. (i) The initial increase in TH activity in the locus coeruleus may be a consequence of the loss of terminals to which the enzyme might be transported (*18*), damage to the mechanism of axonal transport, or both, whereas the later rise in hippocampal TH activity may represent restoration of transport accompanied by an adaptive redirection in enzyme flow. (ii) An increase in the rate of TH synthesis may take place in the locus coeruleus and be followed by the gradual transport of this new enzyme to residual terminals. Whatever the precise mechanism of these changes, it is tempting to speculate that the relatively normal behavior seen in 6-OHDA–treated animals (*8, 19*) results, in part, from this increase in the ability of residual catecholamine-containing nerve terminals to synthesize and release transmitter.

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Selective Inhibition of Glycoprotein and Membrane Protein of Vesicular Stomatitis Virus from Interferon-Treated Cells

Abstract. *A 200-fold inhibition in the titer of infectious vesicular stomatitis virus (VSV) was produced in cultures of Ly cells treated with 30 reference units of interferon per milliliter. Virus particle production, as measured by VSV particle-associated transcriptase, or nucleocapsid protein was inhibited by a maximum of tenfold. The glycoprotein and membrane protein content was reduced in VSV derived from interferon-treated cells. Thus interferon-treated cells may have produced VSV particles with low infectivity, which may be related to the reduced amount of glycoprotein incorporated into such particles. These findings resemble those reported in interferon-treated cells infected with murine leukemia viruses.*

Interferon (IF) inhibits the replication of many viruses. In most systems studied, virus-directed translation or transcription was inhibited (*1*); however, an unusual inhibitory effect of IF treatment on the replication of RNA tumor viruses has been described (*2*). Inhibition of virus production did not correlate with inhibition of the accumulation of viral RNA (*3*) or of viral proteins (*4*). In IF-treated AKR cells production of both endogenous murine leukemia virus (MLV) particles and infectious MLV is decreased, although the intracellular concentration of viral p30 antigen is increased (*4*). Studies on the action of IF in Moloney murine leukemia virus (MMLV) infection of mouse bone marrow and thymus (TB) cells showed that in IF-treated cells there was a 2000-fold decrease in the production of infectious MMLV, a 10- to 20-fold decrease in virus-specific extracellular reverse transcriptase activity, and only a twofold difference in the number of budding viral particles observed on the plasma membrane as determined by scanning electron microscopy (*2*, *5*). In JLS-V5 cells treated with IF the number of cell-associated viral particles was increased, while the infectivity/particle ratio was reduced (*6*). These results suggested that in these systems the release of virus from the plasma membrane or the production of infectious virus particles was inhibited by IF treatment.

One question about this work is whether such findings are restricted to RNA tumor virus systems. We have studied the effect of low concentrations of IF (3 to 30 U/ml) on vesicular stomatitis virus (VSV) infection of Ly cells, in contrast to previous studies, where the IF concentration was higher and VSV-directed transcription or translation was inhibited (*7*). Our results indicated that in Ly cells treated with IF (30 reference units per milliliter) there was an approximately 200-fold reduction in the titer of infectious VSV particle production; however, virus particle production as measured by virion-associated transcriptase or N protein (the structural protein present in VSV in the highest concentration) was inhibited by a maximum of tenfold at this concentration of IF. However, we observed a marked inhibition in glycoprotein (G) and membrane or matrix (M) protein of VSV released from IF-treated cells.

The Indiana strain of VSV (originally obtained from C. Buckler) was plaque-purified and passaged at low multiplicities. Virus titer was assayed as 50 percent tissue culture infectious doses ($TCID_{50}$) by the cytopathic effect (CPE) in BHK or Vero cells with the use of microtiter plates; the titer was 2×10^9 $TCID_{50}$ per milliliter. The Ly mouse cell line (obtained from J. Youngner) is highly sensitive to mouse IF. Mouse IF was prepared and partially purified on an antibody affinity column (*8*). The specific activity of the preparation was greater than 4×10^7 mouse reference units per milligram of protein.

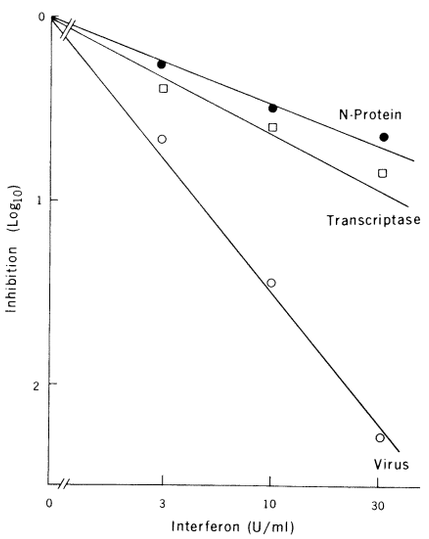

Fig. 1. Effect of IF on virus infectivity, transcriptase, and nucleocapsid (N) protein of released VSV: Ly cells were grown in 150-cm^2 flasks (2×10^7 cells) and treated with 0 to 30 units of IF for 14 hours; the cell monolayers were washed three to four times to remove the residual IF. All the monolayers were then infected with VSV at a multiplicity of five infectious particles per cell and incubated overnight. The supernatants were collected and sedimented at 10,000*g* for 30 minutes to remove cell debris. Portions were saved for infectivity, and the remainder was sedimented at 42,000*g* for 2 hours. The pellets were washed two to three times and used for transcriptase assay (*13*). To study the virion proteins, Ly cells were treated with IF as described above. After virus adsorption and incubation for 5 hours, the monolayers were washed with leucine-free medium and then [^{3}H]leucine (15 to 20 μCi/ml, specific activity 121.1 Ci/mmole) was added to monolayers in special MEM (minimum essential medium) devoid of unlabeled leucine and serum. After 3 hours of incubation, 2 ml of MEM supplemented with 2 percent fetal calf serum was added to each culture and cells were incubated overnight. The supernatants were collected, and virus was centrifuged at 42,000*g* for 2 hours. The pellet was suspended in small volumes of TNE (tris, NaCl, and EDTA) buffer (*p*H 7.2) and virus was further purified by banding on 20 to 60 percent (by weight) equilibrium sucrose gradients for 16 hours at 72,000*g*. Proteins were analyzed by polyacrylamide gel electrophoresis (*14*).

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