this system of neurons may exert subtle modulatory influences and therefore not mediate behavioral changes in a simple one-to-one fashion. The initiation of the behavioral effects of LSD may directly depend on changes in the activity of serotonin-containing neurons, whereas other aspects of its action, such as its peak effects, its duration, and development of tolerance, may depend on factors such as other neurotransmitter systems and synaptic plasticity involving raphe target neurons.

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6-Hydroxydopamine Potentiates Acute Herpes Simplex Virus Infection of the Superior Cervical Ganglion in Mice

Abstract. *Treatment of mice with 6-hydroxydopamine increased herpes simplex virus replication in the superior cervical ganglion while it decreased the subsequent prevalence of latent infection. Preganglionic neurectomy failed to block this effect. These observations suggest that intrinsic neural events modify the outcome of viral infections of the nervous system.*

It is recognized that each cell type in the nervous system may differ in viral susceptibility; however, individual cells within the nervous system are usually assumed to be ontogenetically fixed in their response to infection (*1*). To account for variability in the outcome of infection, attention has been focused almost exclusively on factors extrinsic to the nervous system, such as the dose and neurotropism of the infecting viral strain, the access of virus to neural tissue, and immunologically specific and nonspecific systemic host defenses, while little effort has been directed at defining whether changes in the intrinsic metabolic or functional state of neural cells might influence the character of nervous system viral infection (*1*). To explore this question, I have used a model of herpes simplex virus (HSV) infection of the superior cervical ganglion (SCG) of the autonomic nervous system, and now report that treatment of mice with 6-hydroxydopamine (6-OHDA), a drug that selectively injures adrenergic nerves (*2*), markedly alters the course of infection in this ganglion.

Infection of the SGC occurs after inoculation of the ipsilateral eye with HSV, and the virus reaches the ganglion principally over neural pathways (*3, 4*). The ganglion infection is conveniently considered in two phases: an acute phase, lasting less than 10 days, in which active HSV replication can be monitored by measuring viral titers in homogenates of the ganglion, and a latent phase, during which infectious virus is no longer detected in ganglion homogenates, but the persistence of the viral genome can be demonstrated when virus is reexpressed in ganglia assayed by explantation-cocultivation techniques (*3, 4*). In all aspects tested, SCG infection closely resembles experimental sensory ganglion infection with HSV (*5*).

Systemically administered 6-OHDA is selectively taken up by and destroys adrenergic nerve terminals, resulting in widespread sympathectomy (*2*). In the mature animal, neuronal cell bodies survive and the damaged nerve terminals regenerate. The drug 6-OHDA was selected for this study in order to extend the observation that surgical postganglionic neurectomy, when performed after virus reaches the ganglion, augments HSV replication in the SCG (*4*). As an experimental tool, 6-OHDA offers several advantages over surgical neurectomy in avoiding operative trauma and producing a more uniform and circumscribed lesion.

Four- to six-week-old BALB/c female mice (Charles River) were infected by unilateral intraocular inoculation of 8×10^4 plaque-forming units (PFU) of the F strain of HSV type 1. Methods of virus preparation, inoculation, and assay of ganglia and eyes ipsilateral to the side of inoculation by homogenization and explantation have been described (*3, 4*). 6-Hydroxydopamine hydrobromide (Regis; 250 mg/kg) was dissolved in 0.9 percent sodium chloride containing ascorbic acid (Calbiochem; 0.8 mg/ml) immediately before intraperitoneal injection; control mice received ascorbate vehicle under identical conditions. Previous studies have shown that passive immunization of mice with antibody to HSV reduces the extent of viral replication in the SCG acutely while actually enhancing the subsequent prevalence of latency (*4*), and that immunization protects the integrity of the ganglion (*6*). In addition, enhancement of virus replication by surgical postganglionic neurectomy was most readily recognized in passively immunized mice in which background virus replication was minimal during the acute phase of infection (*4*). For these reasons, both unimmunized mice and mice passively immunized by intraperitoneal injection of 0.2 ml of rabbit antiserum to HSV possessing a neutralizing antibody titer of > 2000 (given 1 day after intraocular HSV challenge) were used in the present studies.

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Administration of 6-OHDA to mice 2 days after they received intraocular injections of HSV markedly altered the course of acute SCG infection. In unimmunized mice (Fig. 1a), SCG infection in drug-treated and control groups was similar on days 3 and 4 after inoculation, but then diverged, with mean viral titers declining to below 10^2 PFU per ganglion on day 5 in control mice, whereas in 6-OHDA-treated animals, titers rose to above 10^4 PFU per ganglion. The influence of 6-OHDA was even more striking in the SCG of immunized mice (Fig. 1b) in which negligible viral titers were detected in ascorbate-injected controls, whereas in the drug-treated group peak viral titers on day 5 again exceeded 10^4 PFU per ganglion. Virus replication in the trigeminal ganglion (TG), a sensory ganglion which also becomes infected after intraocular HSV inoculation, and in the eye, at the site of virus inoculation, were also measured, in order to assess whether the effect of 6-OHDA on HSV replication was limited to the SCG. In neither of these tissues (Fig. 1, c to f) was there a significant difference in HSV replication between 6-OHDA-treated and control mice.

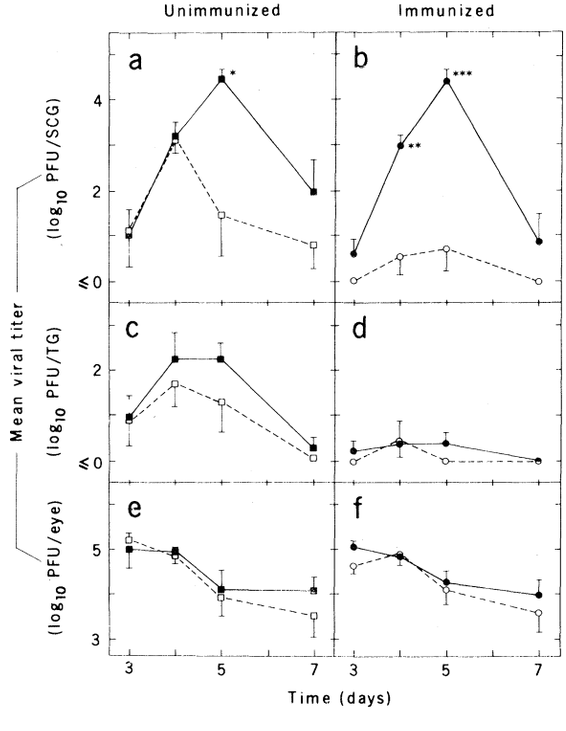
The effect of 6-OHDA on both acute and latent HSV infection depended on the timing of drug administration in relation to virus inoculation. Acute infection was assessed by HSV titers of SCG homogenates from immunized mice killed 5 days after inoculation; infection was augmented when 6-OHDA was given between 2 days before and 2 days after intraocular HSV challenge, whereas less active viral replication occurred when the drug was given earlier (Fig. 2). In contrast, latent infection, assayed by explantation of ganglia from mice killed 25 days after HSV inoculation, was not diminished in immunized mice treated with 6-OHDA 7 days before inoculation but was progressively reduced in mice receiving the drug 2 days before and 2 days after inoculation (Fig. 2). This apparent reciprocal relationship between viral titers and the subsequent prevalence of latency suggests that augmented acute infection and reduced latency are concomitant, temporally circumscribed effects of 6-OHDA on SCG infection. The fact that 6-OHDA given 7 days before virus challenge did not reduce latent infection also indicates that treatment with this drug differs from surgical postganglionic neurectomy carried out before virus inoculation, which has been shown to prevent SCG infection (*4*).

To determine whether the potentiating effect of 6-OHDA on HSV replication is exerted directly on the SCG or mediated by central (presynaptic) neural events, we subjected another group of mice to preganglionic nerve section 2 days before challenging them with intraocular injections of HSV. Preganglionic neurectomy neither modified the augmentation of HSV replication induced in immunized mice by 6-OHDA nor increased productive infection in the absence of drug treatment (Table 1), demonstrating that the increased HSV replication asso-

Table 1. Effect of preganglionic nerve section on 6-OHDA-induced augmentation of SCG infection in immunized mice. Preganglionic neurectomy and sham operations were carried out on mice under pentobarbital anesthesia with the aid of a dissecting microscope 2 days before intraocular HSV inoculation. The preganglionic nerve trunk was severed approximately 2 mm caudal to the SCG; in mice with sham operations the SCG and nerve trunk were identified but left intact. All mice were passively immunized by intraperitoneal injection of antiserum to HSV 1 day after virus inoculation; they were injected with either 6-OHDA or ascorbate vehicle 1 day later and were killed for assay of ipsilateral ganglion homogenates 5 days after inoculation. Each of the four experimental groups contained five mice.

Drug treatment	Operation	Mean viral titers* ($\log_{10}$ PFU/SCG)
6-OHDA	Preganglionic neurectomy	3.9 ± 0.3
6-OHDA	Sham	4.1 ± 0.3
Ascorbate	Preganglionic neurectomy	0
Ascorbate	Sham	0

*The results are expressed as means ± standard error. There was no significant difference by Student's *t*-test between neurectomized and sham-operated 6-OHDA-treated mice or between neurectomized and sham-operated ascorbate-treated animals.



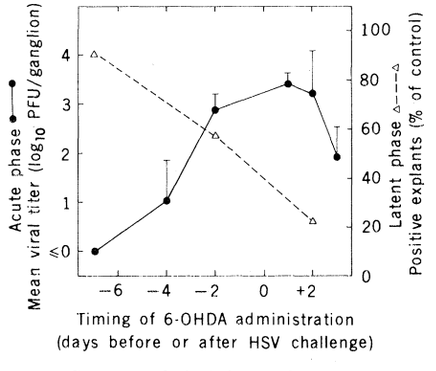


Fig. 1 (left). Effect of 6-OHDA on the course of acute SCG, TG, and eye infections in unimmunized and immunized mice. Mice either received normal rabbit serum (a, c, and e) or were immunized with rabbit antiserum to HSV (b, d, and f) 1 day after they received intraocular injections of HSV. A single injection of 6-OHDA (■ and ●) or ascorbate vehicle (□ and ○) was given 2 days after virus inoculation. Each point represents the $\log_{10}$ mean ± standard error of the mean (S.E.M.) of viral titers of homogenates prepared from the SCG (a and b), TG (c and d), or eyes (e and f) of five or more mice killed at the designated intervals after ipsilateral intraocular HSV inoculation. Significant differences between 6-OHDA-treated mice and ascorbate-treated controls computed by Student's *t*-test are shown: $^*P < .05$, $^{**}P < .01$, $^{***}P < .001$. Fig. 2 (right). Effect of timing of 6-OHDA on acute and latent SCG infection. All mice were passively immunized with antiserum to HSV 1 day after intraocular HSV inoculation; a single injection of 6-OHDA was administered at various times before and after virus inoculation as indicated. The magnitude of acute infection was assessed by $\log_{10}$ mean viral titers (± S.E.M.) of ganglia from five or more mice killed 5 days after ipsilateral intraocular HSV inoculation. Mice were killed 25 days after virus inoculation for assay of latent infection by SCG explantation, and the results are given as the percentage of explants from 6-OHDA-treated mice positive for virus compared to the explants of control animals; each point represents the results of ganglion assay from ten or more drug-treated mice and a similar number of control mice.

ciated with 6-OHDA treatment was not induced transsynaptically.

The most likely explanation for our results is that 6-OHDA directly influenced HSV replication in ganglion cells. Alternative explanations, such as that the drug increased access of virus to the ganglion or interfered with immune defenses that limit intraganglionic viral spread, appear less likely in the light of the observations that drug-induced HSV replication was little influenced by antibody to HSV, and that viral replication in the TG and eye were not significantly altered by drug treatment.

In untreated animals, the outcome of HSV infection of individual ganglion cells is variable. It may be hypothesized that when virus reaches the ganglion cell, it can either actively replicate or become latent (*4, 7*). The factors influencing the outcome are poorly understood. Immunization appears to favor latent infection over active viral replication; whether this reflects a direct modulation of HSV replication by antiviral antibody (*8*) or is exerted by indirect means remains to be determined. In contrast, 6-OHDA exerts an effect opposite to that of immunization, markedly augmenting HSV replication and reducing latency. Furthermore, 6-OHDA overrides the protection conferred by passive immunization, inducing a greater than 1000-fold increase in viral titers while also reducing subsequent latent infection. The concomitant increase in acute HSV replication and decrease in latency following 6-OHDA treatment suggests that cell lysis accompanies augmented productive infection, thereby precluding a role for these infected cells as reservoirs for latent virus.

Further studies are needed to define how injury to terminal axons is communicated to the neuronal parakaryon (*9*), and what cellular metabolic changes underlie the ensuing increased permissiveness for HSV replication. Whether other viral infections may be similarly modified by intrinsic metabolic changes in neural cells or whether, indeed, infection of the central nervous system may be influenced in a manner similar to the SCG also remains to be established. In the case of HSV, it can be speculated that disturbances of peripheral sensory axon terminals might trigger recurrent epithelial eruptions (*5, 10*), while alterations of central nervous system neurons by toxic or physiologic influences, through mechanisms similar to those involved in the 6-OHDA effect on the SCG, might play a decisive role in the pathogenesis of the fulminant, sporadic encephalitis caused by this virus (*11*). If such intrinsic neural mechanisms prove to be important in determining the outcome of human infections, an avenue of therapeutics directed at countering such potentiating influences may warrant future exploration.

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Normal Levels of 5′-Nucleotidase Activity in Lymphocytes from Patients with X-Linked Agammaglobulinemia

Edwards *et al.* (*1*) reported a relative deficiency of 5′-nucleotidase (E.C. 3.1.3.5) in peripheral blood lymphocytes of patients with congenital agammaglobulinemia. However, the cell populations tested, which were mononuclear cell preparations obtained after Ficoll-Hypaque sedimentation, contained both lymphocytes and monocytes. We have found that patients with agammaglobulinemia have considerably increased numbers of monocytes. Human monocytes lack 5′-nucleotidase, acquiring this enzyme activity only after overnight culture (*2, 3*). It seems likely that a deficiency in 5′-nucleotidase in mononuclear cells from patients with congenital agammaglobulinemia could be accounted for by the relative increase in monocytes in the mononuclear cell preparations from these individuals.

To test this hypothesis, we examined cells from four patients with a primary diagnosis of congenital agammaglobulinemia (serum immunoglobulin G < 50 mg per 100 ml, immunoglobulins M and A undetectable). These patients also had affected maternal male relatives, permitting the diagnosis of X-linked agammaglobulinemia. Laboratory studies have demonstrated that these patients lack B lymphocytes. Peripheral blood was treated with 10 m*M* EDTA to prevent coagulation, and mononuclear cells were separated by Ficoll-Hypaque gradient centrifugation. Ficoll-Hypaque

Table 1. Differential cell counts of peripheral blood. Figures are percentages of 200 cells counted, as determined by differential cell count of Wright's stained peripheral blood smear. Values for normal individuals are derived from differential counts of five healthy individuals.

Patient	Monocytes	Lymphocytes	Granulocytes
1	25	36	27
2	28	44	26
3	22	21	56
4	24	22	47
Normal subjects	1 to 6	27 to 45	47 to 59

Table 2. Specific activity of 5′-nucleotidase in monocytes or lymphocytes from individuals with agammaglobulinemia. The data are expressed as nanomoles per minute per milligram of protein. N.D., not determined.

Subject	Monocytes*	Lymphocytes
Patients with agammaglobulinemia		
1	< 0.190	0.745
2	< 0.060	1.827
3	< 0.313	1.326
4	< 0.057	0.358
Mean†	< 0.155 ± 0.122	1.064 ± 0.646
Normal subjects		
1	N.D.	0.246
2	N.D.	1.178
3	< 0.193	2.238
4	< 0.256	2.356
5	< 0.342	2.049
6	< 0.223	3.336
Mean†	< 0.254 ± 0.064	1.901 ± 1.064

*Specific activities for monocytes are maxium activities, which could have been present in the samples assayed, and reflect the sensitivity of the assay procedure. In most cases, samples of monocyte lysates were not distinguishable from control blanks. † Plus or minus standard deviation.

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