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Locomotor Effects of Catecholaminergic Drugs on Herpes-Infected Mice

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SEEGAL, R. F., E. SIKORA AND J. HOTCHIN. *Locomotor effects of catecholaminergic drugs on herpes-infected mice.* PHARMAC. BIOCHEM. BEHAV. 12(1) 61–66, 1980.—Changes in spontaneous, amphetamine (AMP) and apomorphine (APO) induced locomotor activity were used to assess the effects of central nervous system (CNS) infection with herpes type 1 virus. A dual herpesvirus inoculation procedure was used in which the animals received an immunizing footpad inoculation followed at 2 weeks by an identical intracerebral challenge. Four weeks later the animals were tested with intraperitoneal injections of saline or d-l-amphetamine (0.5 and 2.0 mg/kg). When footpad herpes-virus was given via one or two injections, it had no effect on spontaneous or AMP induced activity. When food-pad-intracerebral herpes mice were tested 28–33 days post intracerebral inoculation, they demonstrated depressed AMP-induced but not spontaneous activity. AMP at a dosage of 5.0 mg/kg overcame the herpesvirus blockage of 0.5 and 2.0 mg/kg AMP induced activity. Intraperitoneal injection of APO in day 3 post-IC animals produced less suppression of activity in the virus group than in the controls. These results suggest that non-fatal CNS herpes infection produces hypoactivity, in contrast to the hyperactivity during acute fatal CNS herpes encephalitis (Lycke & Roos, 1975), and that the effect may be due to alterations in postsynaptic receptor sensitivity.

Amphetamine Apomorphine Herpes Simplex virus Locomotor activity

ACUTE infection of the central nervous system (CNS) with herpes simplex type 1 virus (HSV) has been shown to alter locomotor activity and aggression of experimental mice [2]. Furthermore, acute CNS infection with HSV alters both levels and rates of synthesis of dopamine, norepinephrine and 5-hydroxytryptophan following intracerebral (IC) inoculation [1, 2, 4, 5].

Indeed, subacute or even subclinical HSV encephalitis of man may be a cause of abnormal behavior, which presents as a psychosis [3,10]. These observations and other data have led some reviewers to conclude that slowly acting viruses may be a significant cause of some human psychiatric disorders [15,16].

However, to our knowledge there have been no animal studies that have examined longer term effects of HSV infection on behavior. The development of such an animal model would not only more closely match the temporal characteristics of the sub-acute human disease, but would also provide a means of examining long-term interactions between pharmacological agents and the ubiquitous HSV.

We have employed a method in which IC HSV inoculation of mice follows by two weeks a prior immunizing footpad (FP) inoculation. This technique provides substantial protection against the usually lethal effects of the IC virus. Thus, we are able to study the effects of CNS herpes infection in essentially all inoculated mice rather than only in a few survivors of severe, acute infection.

Earlier results showed that for 3–8 days after IC inoculation of herpes virus into FP-immunized mice there was an attenuation of both spontaneous and d-l-amphetamine-induced locomotor activity [9]. In contrast, acute IC only mice demonstrated hyperactivity 3–4 days post IC in a manner and time course similar to that previously reported [2]. In the present study, we extended these observations to longer time intervals and employed a direct dopamine agonist to further analyze infection-induced changes in behavior.

METHOD

Animals

Experimental subjects were male Nya: NYLAR mice from the breeding facilities of the New York State Department of Health. These mice were maintained on a 13 hr light-11 hr dark cycle and were group housed nine to a cage with food and water ad lib. The light cycle began at 0700 local time and the testing was performed between 0900 hr and 1200 hr. At the time of inoculation all mice weighed 8–10 g. Each animal was weighed twice weekly during the course of the experiment.

Virus

The herpes simplex virus type 1 was a human isolate which had been passed twice in human amnion and once in human embryonic lung. The stock virus had an IC titer of 9.9×10^5 mouse LD_{50}/ml and at a 10^{-1} dilution produced death in approximately 14% of the animals receiving FP inoculation. Plaque assay in BHK-21/13s agarose suspension [8] showed a titer of 1.1×10^6 plaque-forming units/ml.

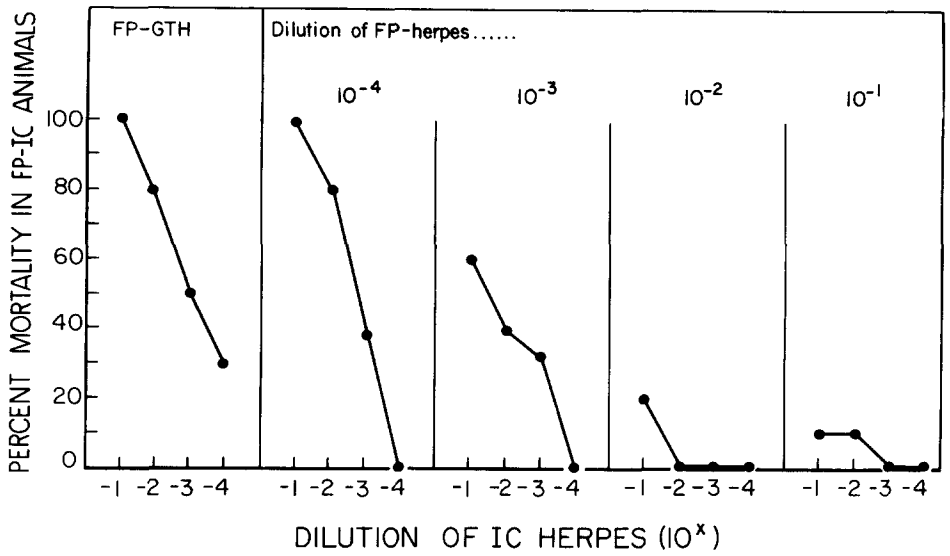

FIG. 1. Percent mortality in mice following FP-IC titration of HSV. Mice received either GTH or HSV followed at two weeks by an IC challenge. HSV dilutions of 10^{-1} through 10^{-4} were employed (N=10/titration sub-group).

For Experiment 5, either undiluted or a 10^{-2} dilution of HSV was inactivated by heating it to 60°C for one hour.

Diluent

0.05% gelatin in tris buffered Hank's solution, ph 7.2 (GTH) was used both as the diluent for virus titration and as the fluid for virus control inoculation for all experiments except No. 5.

Drug Injection

d-l-Amphetamine sulfate (Smith, Kline and French) was made up in physiologic saline at concentrations of 0.5 or 2.0 mg/4 ml and stored at −20°C in 4-ml aliquots. Fifteen minutes before testing, each mouse received an intraperitoneal (IP) injection of the d-l-amphetamine solution at a dosage of 4 ml/kg body weight, yielding an effective dose of 0.5 or 2.0 mg/kg.

Apparatus

Animals were tested in tilt-cage activity monitors designed and built in the laboratory. These were shown to be sensitive to the movement of animals weighing 10 g or more and yielded high correlations between observer scoring of cage crossing and electrical scoring from microswitches closed by the tilt cages. Switch closures were monitored by and recorded on programs written for the BRS/LVE Interact system interfacing a Data General Nova 210 computer.

Experimental Design

Virus inoculation. Based on multiple FP-IC titrations, we have determined that a FP inoculation of a 10^{-2} dilution of the stock virus followed at two weeks by an identical IC dose of virus yielded minimum FP deaths (<2%) and maximum protection (>95%) against subsequent IC challenge. Evidence that animals receiving HSV FP are immune is presented in Fig. 1—it is clear that FP inoculation induces substantial immunity and protection against increasing amounts of virus administered as an IC challenge.

The experimental and control mice received two inoculations; the first was into the left hind FP; the second inoculation 14 days later was made by the IC route. Thirty-six randomly selected mice, designated as experimental, received 0.03 ml of a 10^{-2} dilution of virus in GTH containing approximately 330 PFU; 18 additional mice received control diluent only. Fourteen days later, half (18) of the FP-herpes inoculated mice received an identical IC dose of herpes, while the others received GTH. The control mice each received an equal volume IC inoculation of GTH. These three groups of 18 animals were designated FP-IC herpes, FP herpes-IC GTH, and FP-IC GTH.

For Experiment 1, testing was carried out in a 6-day sequence 4 weeks after the IC inoculation. All animals were tested for 30 min on each test. During the 30-min test period, illumination (970 lux) was provided on alternate days (i.e., animals were tested in the light and the dark). On a given test day, all animals received the same level of amphetamine (or saline) determined by the random selection of sequences from a Latin Square table.

RESULTS

Experiment 1

Effects of amphetamine on FP-IC herpes-infected mice 4 weeks after IC virus (Fig. 2). One month post IC, FP-IC herpes mice exposed to the 2.0 mg level of amphetamine were the least active of the virus or control injection groups. Analysis of the activity of mice given saline only indicated that the virus no longer significantly decreased activity $F(2,51)=0.73$, $p<0.25$, independent of drug effect. Amphetamine increased activity $F(2,102)=432.5$, $p<0.001$, although the effect was not uniform across virus treatment groups resulting in a significant virus × drug interaction $F(4,102)=7.10$, $p<0.001$, in the form of a lowered response of FP-IC herpes mice to the amphetamine-induced activity.

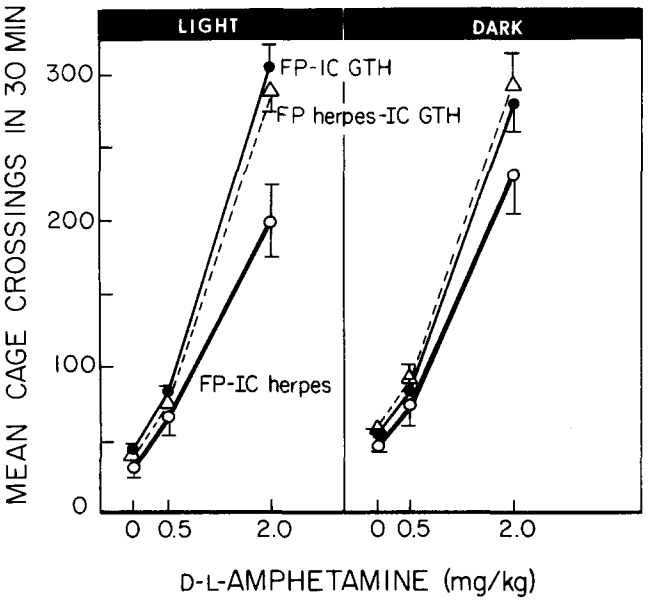

FIG. 2. Mean number of cage crossings (±SEM) in 30 minutes for FP-IC inoculated mice—Days 28–33 post IC: FP-IC herpes (experimental, N=18); FP herpes-IC GTH (Virus control, N=18); FP-IC GTH (injection control, N=18). All mice were exposed to all levels of drugs and tested on alternate days in the light and dark.

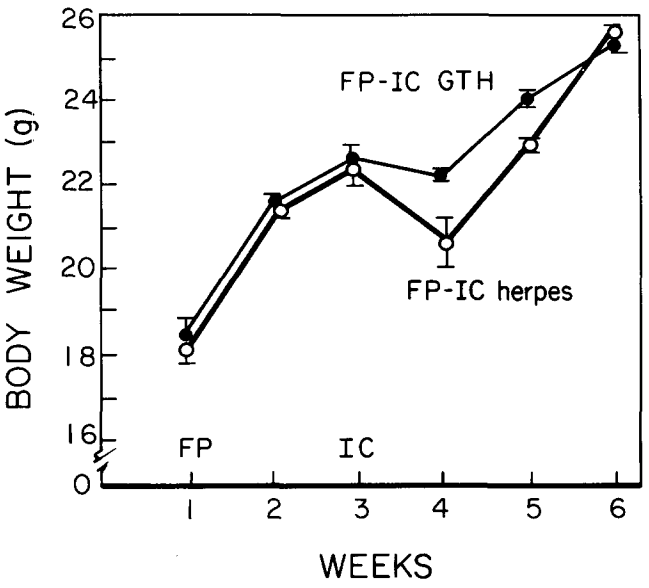

FIG. 4. Weekly body weight determination for FP-IC herpes (experimental) and FP-IC GTH (control) mice.

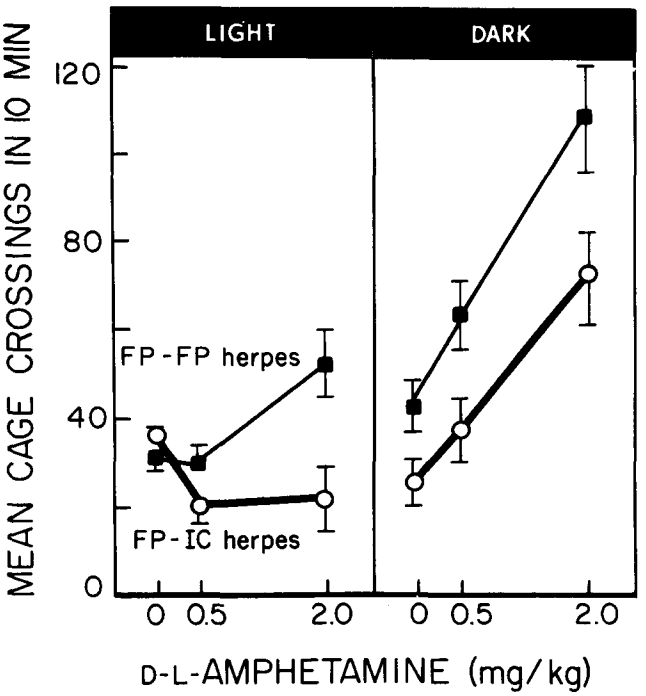

FIG. 3. Mean number of cage crossings (±SEM) per 10 minutes. For FP-IC herpes inoculated mice—Days 3–9 post IC, N=9. Dual FP-FP herpes inoculated mice—Days 3–9 post FP-FP, N=27. All mice were exposed to all levels of drug and tested on alternate days in light and dark.

Experiment 2

Effect of dual FP herpes inoculations on locomotor activity. Because analysis of data from Experiment 1 indicated no effect of a single peripheral (FP) inoculation on behavior, it was decided to discontinue the virus injection control group previously designated as FP herpes-IC GTH.

In Experiment 1, the experimental mice had received two inoculations of herpes, whereas the control groups received only a single virus injection. Hence, the question arose as to whether the major finding of the previous study (an attenuated response to amphetamine induced by IC herpes virus) was due to central herpes infection or to multiple inoculations. To answer that question, mice given two FP inoculations of herpes Type 1 virus at 2-week intervals were compared with mice that received FP and IC inoculations of identical dose and timing.

An unequal N analysis of variance was used to maximize the number of experimental subjects. Of the thirty-six 8–10 g mice used in this experiment, 27 received two FP herpes inoculations separated by 2 weeks; 9 mice received FP-IC herpes inoculations at the same interval. All inoculations were given in the same time sequence and dosage as in Experiment 1. The animals were tested on days 3–8 post IC.

Dual FP-FP herpes mice showed amphetamine dose response curves which were very similar to those obtained with control vehicle injected mice. In contrast, the FP-IC herpes mice continued to demonstrate an attentuated response to d-l-amphetamine (Fig. 3), confirmed by an unequal N analysis of variance. The FP-FP herpes were not only significantly elevated when compared with the FP-IC animals $F(1,34)=7.42, p<0.05$, but analysis also demonstrated a significant virus × drug interaction $F(2,68)=4.74, p<0.05$, again (as in Experiment 1) indicating the inhibitory effects central HSV infection has on amphetamine-induced locomotor activity. Further, albeit indirect evidence that peripheral (FP) exposure to herpesvirus is not capable of producing behavioral change is presented in (Fig. 4). Animals given FP herpes did not show any alteration in growth

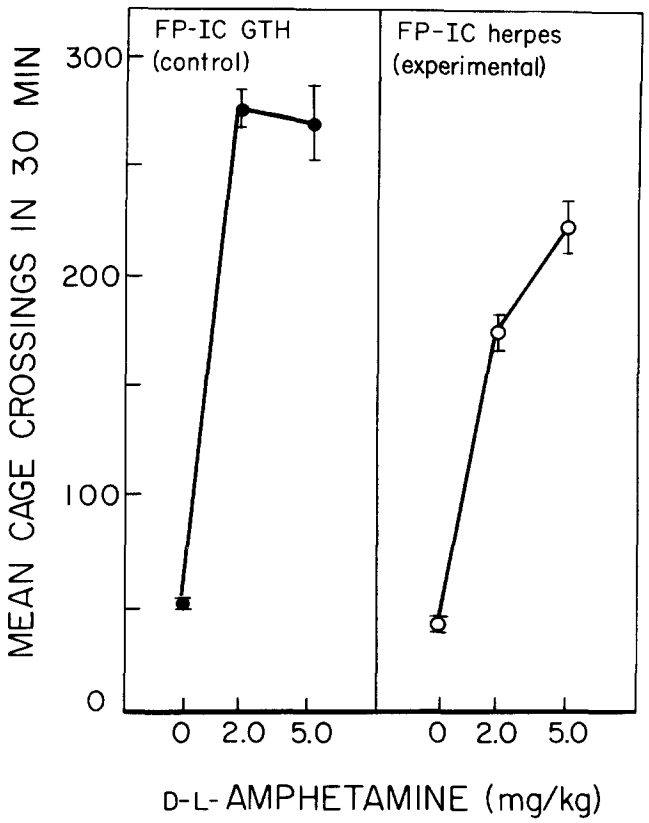

FIG. 5. Mean number of cage crossings (±SEM) in 30 minutes. For FP-IC herpes mice Days 3–5 post-IC: (experimental, N=18). FP-IC herpes mice Days 3–5 post-IC; (experimental, N=18). FP-IC GTH (control, N=9). All mice were exposed to all levels of the drug.

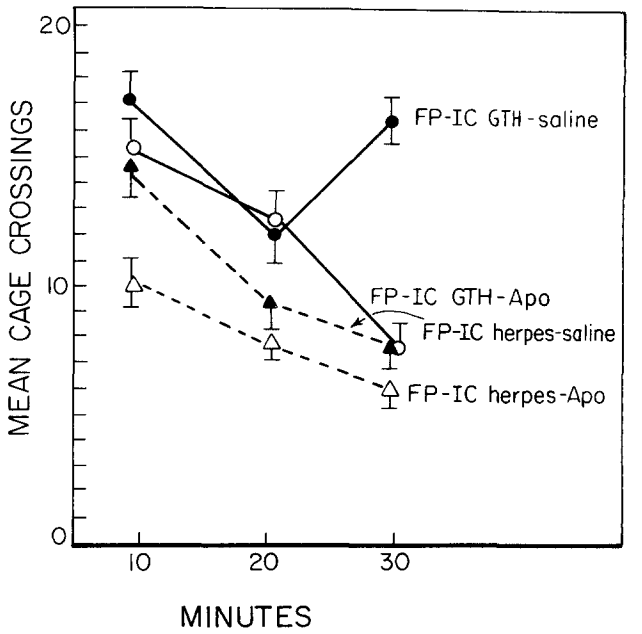

FIG. 6. Mean number of cage crossings (±SEM) in 10 minute recording intervals for FP-IC herpes, N=18 and FP-IC GTH mice, N=18, Days 3–9 post IC. All animals received a single IP injection of apomorphine (4 mg/kg) and were tested in the dark.

(compared to FP-GTH mice) until exposed to central virus infection. At 3 weeks post IC, these mice no longer differed in body weight from the GTH control mice.

Experiment 3

Effects of a single large dose of amphetamine on spontaneous locomotor activity. The difference in locomotor activity between the FP-IC GTH and FP-IC HSV mice observed at the 2.0 mg/kg level of amphetamine might argue that the capability of centrally infected animals to locomote at high rates is limited physically rather than by a disturbance of a central process. In other words, the attenuated locomotor response to amphetamine, rather than representing a central response, might reflect an inability of infected mice to locomote above a certain rate. This hypothesis was tested by exposing FP-IC herpes and FP-IC GTH mice to larger doses of amphetamine than used previously. If the locomotor activity seen at this higher level of amphetamine were heightened, the case for a physical restraint on locomotor activity would be weakened and that for an attenuated central response to amphetamine would be strengthened.

Twenty-seven 8-week-old Nya:NYLAR males were used as experimental and control subjects: 9 as FP-IC GTH controls and 18 as FP-IC herpes. On day 3 post IC, all mice received IP injections of either physiologic saline (4 ml/kg) or d-l-amphetamine 2.0 or (5 mg/kg) 15 min before being placed in the tilt cage. Tests were conducted in the dark on successive days so that each animal was tested under each drug level (saline, 2.0 and 5.0 mg/kg).

FP-IC herpes mice were able to locomote at significantly higher rates at the 5.0 mg level of amphetamine than at the lower (2.0 mg) level of the drug (Fig. 5). Differences in locomotor activity between centrally infected herpes experimental and control mice that existed at the lower dose of amphetamine disappeared at the higher dose ($t=1.59$, $df=34$, $p<0.10$).

Experiment 4

Effects of apomorphine on locomotor activity of herpes-infected and control mice. To aid in further determining the dysfunctions induced by central herpes infection, a comparison was made between amphetamine (an indirect presynaptic releaser of catecholamines) and apomorphine (a direct postsynaptic dopamine agonist). Thirty-six 10–12 g male mice were used with half randomly chosen to receive the standard herpes FP-IC regimen while the remaining animals received FP-IC inoculation of the control vehicle, GTH; testing occurred on Day 3 post-IC inoculation.

A three-factor, repeated measures analysis of variance of the data from the apomorphine experiment showed that virus infection and apomorphine significantly depressed locomotor activity when compared with appropriate controls, respectively (Virus vs. GTH, $F(1,34)=8.83$, $p<0.01$) (apomorphine vs. saline, $F(1,34)=9.14$, $p<0.01$) (Fig. 6). Furthermore, there was a significant drug × time interval interaction $F(2,68)=6.50$, $p<0.01$, and virus × drug × interval interaction. These two drug interactions demonstrated not only a change in the effect of the drug over time (a continuing and deepening suppression of locomotor activity) but also a differential effect across virus treatment groups

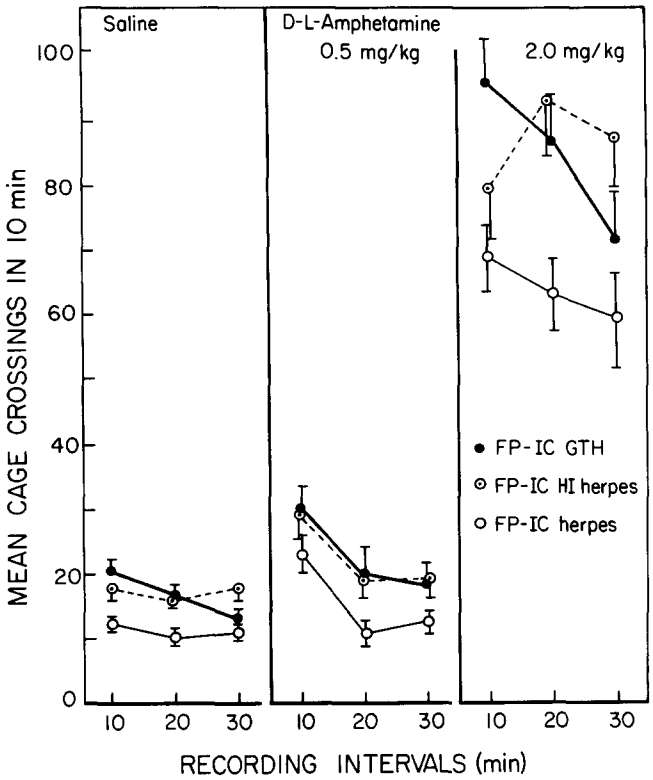

FIG. 7. Mean number of cage crossings (±SEM) in 10 minute recording intervals for FP-IC GTH, FP-IC HSV or FP-IC HIHSV (N=16 for all sub-groups). All mice were exposed to all levels of drug and were tested on Days 3–5 post-IC.

(there was less suppression of activity over time in the herpes-infected group).

Experiment 5

Effects of heat-inactivated HSV on behavior and immunity to IC challenge with HSV. Although remote, the possibility existed that results observed in the previous experiments were not induced by active replicating virus. To rule out that possibility, Nylar mice received either GTH, 10^{-2} HSV or 10^{-2} heat-inactivated (HI) HSV in the FP, followed at two weeks by an identical IC challenge (e.g. FP-IC GTH, FP-IC HSV, FP-IC HI HSV (N=16/sub-group). These animals received IP inoculations of saline, 0.5 and 2.0 mg/kg d-l-amphetamine and were tested in tilt cage activity monitors on days 3–5 post IC. An examination of Fig. 7 indicates that no significant differences existed in spontaneous and drug-induced activity between the GTH-GTH and HIHSV-HIHSV animals, whereas significant differences existed between these two groups and the HSV-HSV animals. These results were confirmed by a repeated measure analysis of variance indicating significant main effects of virus $F(1,40)=574.76$, $p<0.001$; drug $F(2,80)=245.64$, $p<0.001$ and testing interval $F(2,80)=15.83$, $p<0.001$ as well as a three way interaction of virus, drug and interval $F(4,160)=5.25$, $p<0.001$.

Similarly, FP immunization with heat-inactivated virus was found to provide essentially no protection against subsequent IC challenge with active HSV. Both the mean day of death and the percent mortality in HI HSV FP mice were virtually identical to that in animals receiving 10^{-2} HSV IC only (Table 1).

TABLE 1

PERCENT MORTALITY AND MEAN DAY OF DEATH IN NYLAR MALE MICE EXPOSED TO VARIOUS FOOTPAD (FP) VIRUS TREATMENTS AND IC CHALLENGE 14 DAYS POST-FP

	FP GTH 80%	FP (10^{0} HI-HSV) 80%	FP (10^{-2} HI-HSV) 80%
IC 10^{-2} HSV	4.50 days	4.63 days	4.50 days

DISCUSSION

As previously demonstrated [9], the IC inoculation of a second dose of herpesvirus into FP-herpes-immunized mice clearly causes a reduction of both spontaneous and amphetamine-induced locomotor activity. The dual FP-IC herpesvirus inoculation (Experiment 1) caused long-lasting (4 weeks) changes in locomotor response to amphetamine and thus appears to be the first published account of behavioral change in mice induced by nonfatal, as opposed to acute, fatal herpes infection. In addition to providing protection against the normally lethal IC inoculation (Fig. 1), the FP immunization substantially modifies the response normally produced by a single IC-herpes inoculation. Whereas Lycke *et al.* [1, 2, 3, 4] noted hyperactivity and increased turnover of the catecholaminergic neurotransmitters 3–4 days after injection, we observed hypoactivity and an attenuated response to IP inoculations of d-l-amphetamine for 4 weeks post IC infection. Results obtained in Experiment 5 indicate that the observed effects are only produced by an active viral agent and do not occur when the virus is rendered inactive by heat.

Considering the well-known tendency for herpesvirus to demonstrate latency in the nervous system [7, 10, 17] we may be describing behavioral and neuropharmacological differences between (a) an acute virus infection in a nonimmune host characterized by hyperactivity and increased neurotransmitter turnover, and (b) one in a partially immune host that is subacute or exists in a chronic (low-titered) state accompanied by hypoactivity and decreased sensitivity to amphetamine. However, attempts during this period to isolate replicating virus from the brain by standard mouse titration have so far proved unsuccessful. Alternatively, the 4-week long effects may be due to residual damage caused by the non-lethal encephalitis induced by the IC inoculation. A gradual recovery of function in the FP-IC-herpes inoculated mouse is suggested by the fact that 4 weeks after IC inoculation these herpes mice differed from controls at only the 2.0 mg/kg level of amphetamine (Fig. 2). It would appear that in dual inoculated mice, catecholaminergic neurones subserving the locomotor response to amphetamine are capable of responding in a normal fashion to moderate doses of amphetamine but are either incapable of releasing sufficient quantities of transmitter or responding in a "normal" fashion postsynaptically when stimulated by the higher level of amphetamine.

Although it has been claimed that FP inoculation with

herpes may lead to central infection via centripetal movement of the virus along peripheral axons [6,11] our data suggest either that the FP virus failed to reach key areas of the brain involved in the control of locomotor activity or that it was present in such low titers as not to produce any detectable behavioral or neuropharmacological effect. It is clear from the data that FP inoculation alone played no role in the observed behavioral alterations. Three separate observations strongly suggest that central infection is necessary for behavioral changes to occur: (a) FP-herpes-IC-GTH animals, originally used as controls for virus injection, were virtually identical in behavior to FP-IC-GTH-inoculated controls [9]; (b) body weight curves showed no difference between GTH control and FP herpes experimental mice until the latter mice received IC herpes (Fig. 4); and (c) multiple FP herpes inoculations failed to produce any detectable differences from control vehicle injected animals.

The increase in locomotor activity observed among FP-IC herpes mice given the 5.0 mg/kg dose of d-l-amphetamine strongly argues against a virus-induced muscular or neuromuscular dysfunction that might impose a ceiling on rates of locomotor activity. Instead these results suggest that IC herpes inoculation alters the central neurochemical mechanisms by which amphetamine enhances locomotor activity, perhaps by reducing the sensitivity of or number of active pre- and/or postsynaptic sites.

In contrast to published reports that apomorphine enhances locomotor activity [12,14] we found that apomorphine (4 mg/kg IP) caused a decrease in locomotor activity. This discrepancy may be partially explained by the use of photocell cages in the former studies versus tilt cages in the present study. It is likely that photocell, but not tilt cages, would be sensitive to and record the small movements associated with apomorphine induced stereotypical behavior. In addition, Thornburg and Moore and Strömbom adapted their subjects to the apparatus prior to testing, whereas we did not (a procedure which has been shown to alter apomorphine effects on locomotor activity) [12].

Most important, it was found that apomorphine injection in herpes infected mice led to significant drug × virus and drug × virus × time interval interactions which demonstrated that there was less apomorphine-induced suppression of locomotor activity in the herpes-infected group than in the FP-IC GTH control group.

Thus, we have demonstrated that herpes infection of the CNS, induced by a dual FP-IC inoculation, caused chronic changes in locomotor responses to both amphetamine and apomorphine. Taken together, these results, based on the use of both direct and indirect dopamine receptor agonists, suggest that the observed sequelae of the virus infection are due to an alteration in either the sensitivity of or the number of postsynaptic catecholaminergic sites that control locomotor activity.

Although amphetamine has been shown to interact with the base level of activity of the animal [13] it does not appear to be a factor in this study since amphetamine effects were observed in animals four weeks post-IC, a time at which there were no differences in saline locomotor activity between infected and control animals.

These findings are in keeping with, and tend to reinforce, the indications that nonfatal herpes simplex virus infection of the brain may lead to both acute effects and chronic behavioral sequelae in both animals and man.

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