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Infection of cultured central nervous system neurons with a defective herpes simplex virus 1 vector results in stable expression of *Escherichia coli* β-galactosidase

(transfection of neurons/neuronal physiology/learning and memory/gene therapy)

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ABSTRACT **We have developed a defective herpes simplex virus (HSV) vector system that permits the introduction of virtually any gene into mammalian central nervous system neurons. The prototype vector, pHSVlac, contains a transcription unit that places the *Escherichia coli lacZ* gene under the control of the HSV-1 immediate early 4/5 promoter. pHSVlac was propagated using the HSV-1 temperature-sensitive mutant ts K as helper virus. Infection of rat neurons in primary culture derived from various regions throughout the central nervous system, including spinal cord, cerebellum, thalamus, basal ganglia, hippocampus, occipital cortex, temporal cortex, and frontal cortex, resulted in stable expression of high levels of β-galactosidase for at least 2 weeks, without cell damage. Since other genes can be expressed from pHSVlac, HSV-1 vectors may prove useful for delivery of genes into central nervous system neurons for studies on nervous system physiology or to perform gene therapy for neurological conditions.**

One of the goals of neurobiology is to understand the physiology of neurons at the molecular level. The ability to identify specific neurons and then alter their physiology has proved to be a powerful approach for understanding learning and memory in invertebrates such as *Aplysia* (1). Much has been learned about the physiology of groups of neurons in particular areas of the mammalian cortex, such as the functional organization and receptive field properties of neurons in the primary visual cortex (2). However, the level of analysis in mammalian systems is not as exact as that for invertebrates. The capability to introduce a gene into mammalian neurons *in vivo*, thereby altering the physiology of neurons in a precise and reproducible manner, might assist in the analysis of the physiology of neurons and the mechanism of learning and memory.

In the past, four approaches have been used to deliver DNA into cells: (*i*) microinjection of frog oocytes, (*ii*) transgenic mice, (*iii*) transfection of DNA into cells, and (*iv*) retrovirus vectors (3–6). Unfortunately, none of these methods can deliver a gene directly into postmitotic cells. Therefore, we have developed defective herpes simplex virus 1 (HSV-1) vectors to introduce genes into postmitotic cells such as neurons. HSV-1 vectors have a number of attractive features (7). These include the wide host range of the virus; HSV-1 can infect a large number of cell types in mammals and birds (8). Of note, HSV-1 can infect postmitotic cells, including neurons in adult animals. In neurons, HSV-1 can be maintained indefinitely in a latent state (9). Since latent HSV-1 is dormant, most HSV-1 genes are silent, although at least one gene is expressed (10). Furthermore, several electrophysiological parameters are unchanged in neurons harboring latent HSV-1 (11). Finally, the large [150 kilobases (kb)] genome of HSV-1 suggests that a HSV-1 vector could contain up to several genes.

Our prototype HSV-1 vector, pHSVlac (12), contains the *Escherichia coli lacZ* gene (13) under the control of the HSV-1 immediate early (IE) 4/5 promoter (14), a constitutive promoter that functions in many cell types. The *lacZ* gene encodes a β-galactosidase absent from mammalian cells, thereby providing an assay for gene expression (15, 16); we have previously shown that pHSVlac stably expresses β-galactosidase in cultured rat peripheral neurons (12). pHSVlac was propagated (17) with temperature-sensitive (ts) mutants of HSV-1 (18) and virus was grown at the permissive temperature of 31°C. In turn, expression experiments were performed at 37°C; at this restrictive temperature, the ts mutation blocks the lytic cycle, thereby preventing cell damage. Previously reported HSV-1 vectors were propagated with wild-type HSV-1 and always caused cell death (19, 20).

The *lacZ* gene in pHSVlac can be replaced with virtually any other gene. To alter neuronal physiology, genes encoding components of second messenger systems or neurotransmitter release could be expressed from HSV-1 vectors. Furthermore, it is conceivable that HSV-1 vectors could be used to perform gene therapy in a variety of neurological disorders; for example, delivery of the tyrosine hydroxylase gene into neurons in the substantia nigra or the striatum might be an effective treatment for Parkinson disease.

In this study we demonstrate that pHSVlac expresses β-galactosidase following infection of cultured rat neurons derived from areas throughout the central nervous system (CNS), including spinal cord, cerebellum, thalamus, basal ganglia, hippocampus, occipital cortex, temporal cortex, and frontal cortex. In addition, β-galactosidase is stably expressed in CNS neurons for at least 2 weeks following infection. Furthermore, pHSVlac DNA persists in cells for at least 2 weeks and can be recovered following superinfection with HSV-1.

MATERIALS AND METHODS

Enzymes and Chemicals. The restriction endonucleases *Eco*RI and *Hin*dIII were obtained from New England Biolabs. Genetran was obtained from Plasco (Woburn, MA).

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Abbreviations: HSV-1, herpes simplex virus 1; CNS, central nervous system; ts, temperature-sensitive; IE, immediate early; pfu, plaque-forming units; moi, multiplicity of infection; β-Gal-IR, β-galactosidase-like immunoreactivity; Nf-IR, neurofilament-like immunoreactivity.

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Mouse monoclonal anti-rat neurofilament antibody (SMI-33; ref. 21) was obtained from Sternberger–Meyer (Jarretsville, MD). Rabbit anti-*E. coli* β-galactosidase antibody, fluorescein isothiocyanate-conjugated goat $F(ab')_2$ antibody to mouse $F(ab')_2$, and rhodamine isothiocyanate-conjugated goat $F(ab')_2$ antibody to rabbit $F(ab')_2$ were obtained from Cooper Biomedical. Tissue culture reagents were obtained from GIBCO.

Cell Culture and Viruses. CV1 monkey fibroblasts were grown in Dulbecco's modified minimum essential medium with 10% fetal bovine serum. HSV-1 strain 17 ts K (18) was kindly provided by J. Subak-Sharpe (University of Glasgow, Glasgow, Scotland). Cultures of embryonic mouse spinal cord were kindly provided by Anat Lev (Massachusetts General Hospital, Boston), and initial cultures of neonatal rat hippocampus were kindly provided by Edwin J. Furshpan (Harvard Medical School, Boston). Cultures of hippocampus, basal ganglia, total neocortex (22), cerebellum, thalamus, and cortical areas (23) were prepared as described from 1- to 4-day-old rats. Cultures were prepared on five 13-mm glass coverslips in a 35-mm dish; each glass coverslip was coated with 20 μg of poly(L-lysine). Five days after plating, cultures were treated with 40 μM arabinonucleoside (cytosine arabinoside) for 24 hr to prevent glial overgrowth. Five to 16 days after the arabinonucleoside treatment, cultures were infected with pHSVlac virus; each 35-mm dish contained $\approx 1 \times 10^5$ cells at the time of infection, and ≈10% of the cells were neurons.

Construction of pHSVlac and Packaging into HSV-1 Virus Particles. pHSVlac was constructed (12) and packaged (17) into HSV-1 virus particles as described. Briefly, 1.5×10^5 CV1 cells were seeded on a 60-mm plate. The following day the cells were transfected (5) with a 0.5-ml calcium phosphate coprecipitate containing 1 μg of pHSVlac DNA and 9 μg of salmon sperm DNA. Four hours later the cells were treated with 15% glycerol (24). Following a 24-hr incubation at 37°C, 1.5×10^6 plaque-forming units (pfu) of HSV-1 ts K (18) in 100 μl of medium was added to each plate. After 1 hr at room temperature, an additional 5 ml of medium was added to each plate; following an incubation for 3 days at 31°C, virus was harvested. Virus was subsequently passaged at a 1:2 dilution on CV1 cells at 31°C. Virus was prepared, passaged, and titered as described (25). The titer of the virus stock was 1×10^6 pfu of ts K per ml and 8×10^5 infectious particles of pHSVlac per ml; pHSVlac virus was titered by determining the number of β-galactosidase-positive PC12 cells following infection with increasing amounts of pHSVlac virus (12).

Immunofluorescent Visualization of β-Galactosidase and Neurofilament. Primary cultures of CNS neurons were infected with 0.1 ml of pHSVlac virus stock, incubated for either 24 hr or 2 weeks at 37°C, and fixed with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Immunohistochemistry was performed as described (12); the primary antibodies were rabbit anti-*E. coli* β-galactosidase (1:800 dilution) and mouse monoclonal anti-rat neurofilament (1:800 dilution). (This antibody efficiently reacts with mouse, rat, and human neurofilaments.) The secondary antibodies were fluorescein isothiocyanate-conjugated goat $F(ab')_2$ antibody to mouse $F(ab')_2$ (1:200 dilution) and rhodamine isothiocyanate-conjugated goat $F(ab')_2$ antibody to rabbit $F(ab')_2$ (1:250 dilution). Coverslips were mounted in PBS/glycerol (1:1) containing 0.4% *n*-propyl gallate.

Recovery and Analysis of pHSVlac DNA from Cultured Cells. This procedure has been described in greater detail (12). Cultures of total neocortex were infected with 0.1 ml of pHSVlac virus stock and incubated for 2 weeks at 37°C; cultures were then infected with 5×10^5 pfu of ts K and incubated for 2 days at 31°C. The resulting virus was passaged three times on 2×10^6 CV1 fibroblasts at 31°C to yield virus stocks Cor-1 and Cor-2. CV1 cells (1×10^7) were infected with 5×10^7 pfu of virus (Cor-1, Cor-2, ts K alone, or mock infected) and incubated at 31°C for 24 hr.

Total cellular DNA was prepared as described (26). Five micrograms of DNA, or 2×10^{-4} μg of pHSVlac DNA isolated from *E. coli* HB101 as standard, was digested with 12.5 units of the restriction endonuclease *Eco*RI overnight at 37°C, resolved on 0.7% agarose gels, and transferred to Genetran as described (27). Hybridization was performed as described (27); the probe was the 3.3-kb *Eco*RI–*Hin*dIII fragment from the plasmid pCH110 (13) radiolabeled with ^{32}P (28).

RESULTS

The Vector, pHSVlac. The 8.1-kb defective HSV-1 vector, pHSVlac (12), is schematically represented in Fig. 1. pHSVlac contains three types of genetic elements: (*i*) sequences from pBR322 that allow propagation of pHSVlac in *E. coli*; these include the ampicillin-resistance gene (amp^R) and the ColE1 origin of DNA replication (ori); (*ii*) sequences from HSV-1 that permit packaging of pHSVlac DNA into HSV-1 virus particles; these are the HSV-1 ori_s (a HSV-1 origin of DNA replication) (14) and the HSV-1 a sequence (the packaging site) (29); and (*iii*) a transcription unit, the components of which are the HSV-1 IE 4/5 promoter (14), the intervening sequence following that promoter, the *E. coli lacZ* gene (13), and the simian virus 40 early region poly(A) site (13). Because the *lacZ* gene encodes a β-galactosidase absent from mammalian cells, it permits a simple assay for expression of the transcription unit in pHSVlac.

Expression in Neurons. To determine if pHSVlac could express β-galactosidase in CNS neurons, cultured rat CNS neurons (22, 23) were infected with pHSVlac virus and incubated at 37°C for 24 hr, and an immunofluorescent assay was performed to demonstrate expression of *E. coli* β-galactosidase in neurons. As shown in Fig. 2, phase-positive cultured cells from occipital cortex, temporal cortex, and

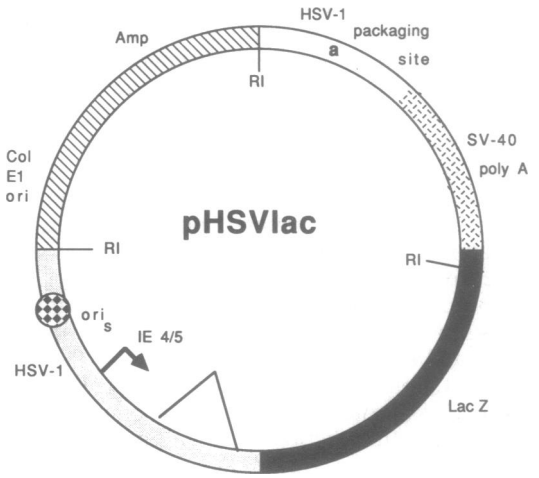

FIG. 1. Structure of pHSVlac. The hatched segment represents sequences from pBR322, the ampicillin-resistance gene, and the ColE1 origin of DNA replication. The clear region contains the HSV-1 a sequence nucleotides 127–1132, the packaging site (29). The crosshatched region contains HSV-1 DNA from the c region nucleotides 47–1066 (14), which contains the following genetic elements: the HSV-1 origin of DNA replication ori_s, represented by a circle filled with small triangles; the HSV-1 IE 4/5 promoter, represented by the arrow; and the intervening sequence following that promoter, represented by the triangle. The black segment contains the *E. coli lacZ* gene (13) and the dotted portion contains the simian virus 40 (SV-40) early region poly(A) site (13). The three *Eco*RI sites are symbolized by RI. The sizes of the *Eco*RI fragments are as follows: the fragment containing the HSV-1 c region and the *lacZ* gene, 4.3 kb; the fragment containing the pBR segment, 2.3 kb; and the fragment containing the HSV-1 a region, 1.5 kb.

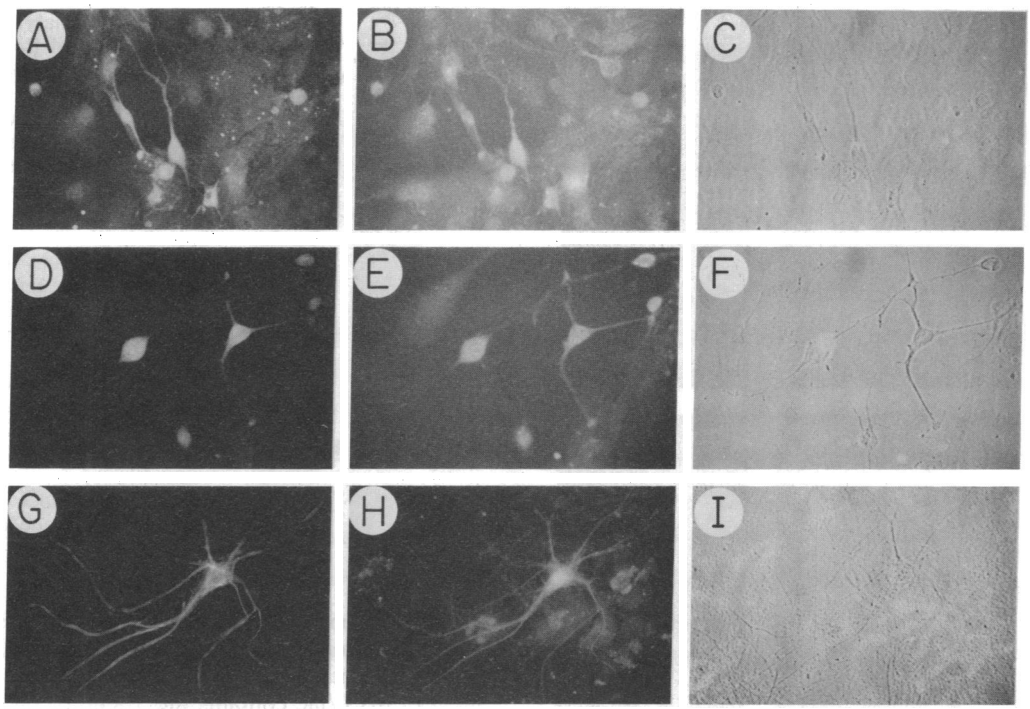

FIG. 2. Immunofluorescent colocalization of β-galactosidase-like immunoreactivity (β-Gal-IR) and neurofilament-like immunoreactivity (Nf-IR) in cultured rat CNS neurons 24 hr after infection with pHSVlac virus. Cultures of CNS neurons were infected with pHSVlac virus and incubated at 37°C for 24 hr. Immunohistochemistry was performed as described (12) using primary antibodies against *E. coli* β-galactosidase and rat neurofilament. β-Gal-IR was visualized with a rhodamine-conjugated secondary antibody and Nf-IR was visualized with a fluorescein-conjugated secondary antibody. The width of each photomicrograph represents 230 μm. (*A*) Nf-IR. (*B*) β-Gal-IR. (*C*) Phase-contrast photomicrograph of the same field as in *A* and *B* from a culture of occipital cortex. (*D*) Nf-IR. (*E*) β-Gal-IR. (*F*) Phase-contrast photomicrograph of the same field as in *D* and *E* from a culture of temporal cortex. (*G*) Nf-IR. (*H*) β-Gal-IR. (*I*) Phase-contrast photomicrograph of the same field as in *G* and *H* from a culture of hippocampus.

hippocampus contained β-Gal-IR and Nf-IR. In these cultures ≈10% of the cells were neurons and between 60% and 70% of the neurons in the microscopic fields examined contained β-Gal-IR. Since the average multiplicity of infection (moi) was 0.8, pHSVlac virus efficiently infected neurons. Some cells contained β-Gal-IR but lacked Nf-IR and had the morphological appearance of glia; these cells will be reported on elsewhere. Furthermore, β-Gal-IR was observed in cells that contained Nf-IR in cultures of spinal cord, cerebellum, thalamus, striatum, and frontal cortex (results not shown). Cultures infected with pHSVlac virus and treated with preimmune primary serum contained background levels of fluorescein and rhodamine fluorescence (results not shown); cultures infected with pHSVlac virus and treated with anti-neurofilament antibody and rabbit preimmune serum followed by the fluorescent-conjugated antibodies contained Nf-IR but no β-Gal-IR. In addition, cultures infected with ts K alone or mock infected, and treated with anti-neurofilament and anti-β-galactosidase antibodies followed by the fluorescent-conjugated secondary antibodies, contained Nf-IR but lacked β-Gal-IR. To demonstrate that pHSVlac could stably express β-galactosidase, CNS cultures were infected with pHSVlac virus, incubated for 2 weeks at 37°C, and then assayed for expression of β-galactosidase. Fig. 3 shows phase-positive cells from total neocortex and hippocampus that contain Nf-IR and β-Gal-IR 2 weeks after infection with pHSVlac virus. Between 60% and 70% of the hippocampal and neocortical neurons in the microscopic fields examined were β-galactosidase positive. One day or two weeks after infection, mock-infected cultures and cultures infected with pHSVlac virus contained similar numbers of neurofilament-positive cells.

Horizontal Transmission of pHSVlac. Neurons containing β-Gal-IR 2 weeks after infection with pHSVlac virus could result either from persistence of pHSVlac DNA in a neuron for 2 weeks or from horizontal transmission of pHSVlac from an infected neuron to an uninfected neuron. If horizontal transmission occurred at a significant frequency, then virtually all cells in a culture would contain pHSVlac DNA and express β-galactosidase, and ts K and pHSVlac virus would be present in the culture medium. In contrast, at least 30% of the cells that contained Nf-IR lacked β-Gal-IR. In addition, 2 weeks after infection of cultures of neocortex, the amounts of pHSVlac and ts K virus in the culture medium were either below our level of detection or very low (Table 1). The low level of virus in the culture medium might be due to release of virus from dead cells during freezing and thawing of medium; dead cells were observed in the medium under the microscope. In contrast, wild-type HSV-1 kills all cells in these cultures in <24 hr. Furthermore, in cultured peripheral nervous system neurons, as well as cultures of differentiated PC-12 rat pheochromocytoma and differentiated N1E-115 mouse neuroblastoma cells, pHSVlac infection results in no detectable ts K or pHSVlac virus in the culture medium 2 weeks after infection (ref. 12; A.I.G., unpublished observations). In summary, although it is not necessarily absent, the rate of horizontal transmission of pHSVlac in neocortical cultures is very low.

Recovery of pHSVlac DNA from Neurons. A strategy was devised to recover pHSVlac DNA from cultured neurons, based on the observation that superinfection of a latently infected neuron results in a lytic infection with the latent genome and the superinfecting genome present in the progeny virus (30). After infection with pHSVlac virus and incubation for 2 weeks, cultures were then infected with ts K alone and incubated for 2 days at 31°C. Total cellular DNA (26) was isolated from the resulting virus stocks and digested with the restriction endonuclease *Eco*RI. To detect pHSVlac

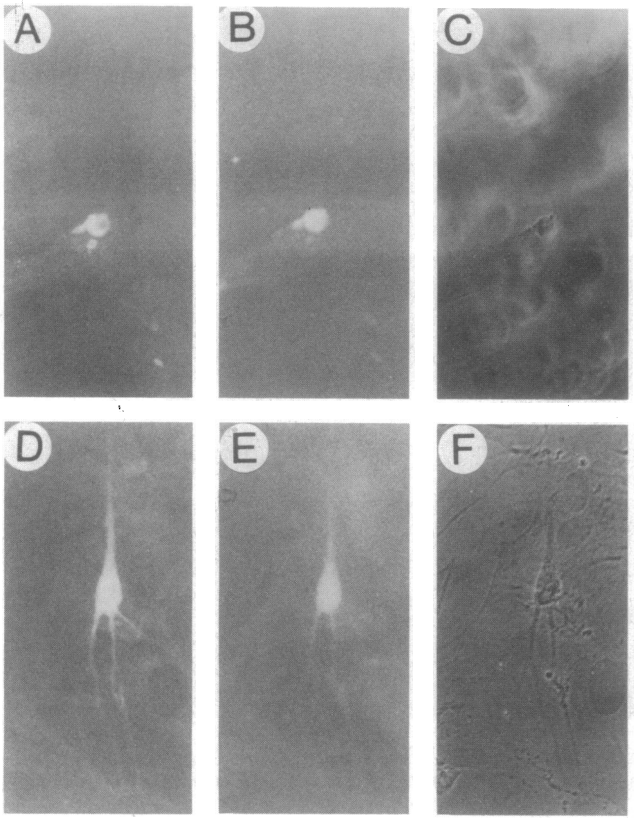

FIG. 3. Immunofluorescent colocalization of β-Gal-IR and Nf-IR in cultured CNS neurons 2 weeks after infection with pHSVlac virus. The experiment was performed as described in the legend to Fig. 2 except cultures were incubated for 2 weeks after infection with pHSVlac virus. The width of each photomicrograph represents 90 μm. (*A*) Nf-IR. (*B*) β-Gal-IR. (*C*) Phase-contrast photomicrograph of the same field as in *A* and *B* from a culture of total neocortex. (*D*) Nf-IR. (*E*) β-Gal-IR. (*F*) Phase-contrast photomicrograph of the same field as in *D* and *E* from a culture of hippocampus.

DNA, Southern analysis (27) was performed; the probe was the 3.3-kb *Eco*RI–*Hin*dIII fragment from the plasmid pCH110 (13). This fragment contains most of the *lacZ* gene, except for 133 base pairs (bp) at the 3′ end (13). As shown in Fig. 1, pHSVlac (12) contains three *Eco*RI sites, one at each end of the pBR segment and a third in the *lacZ* gene 133 bp from the 3′ end of the fragment. As shown in Fig. 4, the 4.3-kb band, which contains most of the transcription unit in pHSVlac, is present in virus obtained from superinfection of neocortical

Table 1. Amount of ts K and pHSVlac virus present in the medium of cultures of total neocortex 2 weeks after infection with pHSVlac virus

Culture	ts K*	pHSVlac†
1	8×10^{2}‡	1×10^{1}
2	$<10^{1}$	4×10^{1}
3	$<10^{1}$	$<10^{1}$
4	$<10^{1}$	$<10^{1}$

Cultures of total neocortex were infected with pHSVlac virus and incubated at 37°C for 2 weeks. Culture medium was removed and frozen and thawed once, and the titer was determined.

*pfu of ts K per ml (25).

†Infectious particles of pHSVlac per ml (ref. 12; A.I.G., unpublished observations).

‡In contrast, a lytic infection of HSV-1 yields several hundred pfu per cell (8); therefore, a productive infection of a culture (1×10^{5} cells) would yield $\approx 1 \times 10^{7}$ pfu of ts K per ml. This experiment was performed three times with four cultures per experiment; ts K virus was detected only once.

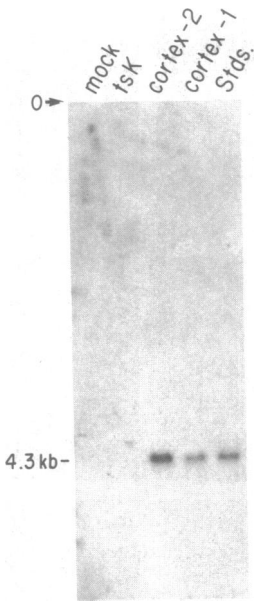

FIG. 4. Analysis of pHSVlac DNA recovered from cultured neocortical cells 2 weeks after infection with pHSVlac virus. Cultures of total neocortex were infected with pHSVlac virus, incubated for 2 weeks at 37°C, and then infected with ts K and incubated for 2 days at 31°C. Total cellular DNA was isolated and subjected to Southern analysis with a probe homologous to the *lacZ* gene. The origin of the gel is marked with an O, and the sizes of the DNA fragments are as shown. Lanes labeled cortex-1 and cortex-2 contain DNA from CV1 cells infected with virus recovered from cultures of neocortex 2 weeks after infection with pHSVlac virus; lanes labeled ts K or mock contain DNA from CV1 cells infected with ts K or mock infected, respectively; and the lane labeled Stds. contains pHSVlac DNA isolated from *E. coli* HB101.

neurons harboring pHSVlac DNA but is absent from ts K alone and from uninfected cells. The 2.3-kb fragment of pHSVlac contains the pBR sequences; and the 1.5-kb fragment of pHSVlac contains the 3′ end of the *lacZ* gene, the simian virus 40 early region poly(A) site, and the HSV-1 a sequence. These two fragments are not homologous to the probe. Finally, we demonstrated that the pHSVlac DNA recovered from persistently infected neurons contained a functional transcription unit: PC12 cells were infected with the virus obtained from superinfection with ts K of neurons harboring pHSVlac DNA; 1–5% of the PC12 cells contained β-galactosidase. We conclude that pHSVlac DNA stably persists in CNS neurons for at least 2 weeks.

DISCUSSION

Until now, the techniques used to deliver genes into mammalian cells (3–6) have been unable to deliver a gene directly into postmitotic cells such as neurons. Therefore, we have developed a defective HSV-1 vector system to deliver genes into neurons (7, 12) by exploiting several features of the virus (8–11): HSV-1 is neurotropic, it can infect postmitotic cells, and in neurons HSV-1 can be maintained indefinitely in the latent state. Infection with pHSVlac virus of neurons in primary culture derived from areas throughout the rat CNS resulted in expression of β-galactosidase without causing cell death. Expression of β-galactosidase in cells containing Nf-IR, a hallmark of neurons, was maintained for at least 2 weeks. The rate of horizontal transmission of pHSVlac was low, shown by low titers of ts K and pHSVlac virus in the culture medium and by the presence of β-galactosidase-negative cells in the cultures. Recovery and analysis of pHSVlac DNA from cells 2 weeks after infection with pHSVlac virus confirmed the presence of pHSVlac DNA. These results extend our previous studies demonstrating infection with pHSVlac virus and expression of β-galactosidase in cultured peripheral neurons (12) and differentiated neuroblastoma and pheochromocytoma cell lines (A.I.G., unpublished observations).

As shown elsewhere (ref. 12; A.I.G., unpublished observations), expression of β-galactosidase from pHSVlac is independent of the helper virus ts K; infection of cells at a low moi of pHSVlac virus (0.10) resulted in expression of β-galactosidase. Addition of up to 10 moi of ts K to such an experiment did not increase either the amount of β-galactosidase or the number of β-galactosidase-positive cells.

Thus, a single HSV-1 particle containing pHSVlac DNA can render a cell β-galactosidase positive.

In this study, pHSVlac DNA was packaged into HSV-1 virus particles using ts K as helper virus. Unfortunately, ts mutants revert to wild type; the reversion frequency for ts K is 9×10^{-5}. Consequently, HSV-1 vectors grown using ts K as helper virus are not suitable for human gene therapy. In contrast, vector DNA packaged into virus particles using a deletion mutant (31) of HSV-1, which cannot revert, might be appropriate for use in humans.

In the latent state, HSV-1 gene expression is limited to a latency-associated transcript (10) and possibly the IE genes (32). Although RNA homologous to the IE genes could not be detected in latently infected neurons using *in situ* hybridization (10), an IE protein has been detected in latently infected neurons using immunofluorescence (32). In addition, IE promoters function constitutively in mitotic fibroblasts; transfection studies have shown that in stably transformed fibroblasts the IE promoters are active (33, 34). Our results demonstrate that the IE 4/5 promoter stably functions in persistently infected CNS neurons and peripheral neurons (12).

In the human CNS, HSV-1 infection occurs preferentially in particular areas (35). In contrast, in the rodent CNS the areas that are infected depend on the particular site of inoculation of wild-type HSV-1 (36). The anatomical path of HSV-1 infection *in vivo* is influenced by the interaction of many complex factors, including the site of initial infection; preferential virus growth in some cell types; the projection sites of neurons; rates of anterograde and retrograde transport of HSV-1; and the immune response, if any, to the virus (9, 35, 36). A productive lytic infection is not necessary for pHSVlac to express β-galactosidase; all that is required is that pHSVlac virus must be able to adsorb to the cell and that pHSVlac DNA must be delivered to the nucleus. Our results demonstrate that these two requirements for β-galactosidase expression are met in cultured neurons derived from areas throughout the CNS, including areas not usually infected by HSV-1. Thus, we conclude that pHSVlac is capable of introducing and expressing a gene in many CNS neurons; however populations of neurons resistant to infection by pHSVlac virus may exist.

Since HSV-1 DNA is found in the nucleus and is transcribed by the cellular DNA-dependent RNA polymerase II (8), expression of β-galactosidase demonstrates that pHSVlac DNA is in the nucleus. Although there is some evidence for integration (37, 38), the physical location of latent HSV-1 DNA in the nucleus of a neuron is not known. It is possible that HSV-1 DNA or pHSVlac DNA could stably persist in an extrachromosomal state since neurons do not divide.

The *lacZ* gene in pHSVlac can be replaced with almost any other gene—for example, genes whose products affect neuronal physiology, including components of second messenger systems or neurotransmitter release (7). Such recombinant HSV-1 vectors could then be used to perturb neuronal physiology to study learning and memory. For example, injection of the enzyme protein kinase C into hippocampal pyramidal neurons has been shown to elicit some properties of long-term potentiation (39). Therefore, introduction of an altered protein kinase C gene into hippocampal neurons *in vivo* might affect long-term potentiation and produce altered performance on learning paradigms.

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