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ARTICLE

Widespread Correction of Lysosomal Storage in the Mucopolysaccharidosis Type VII Mouse Brain with a Herpes Simplex Virus Type 1 Vector Expressing β-Glucuronidase

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We have inoculated a herpes simplex virus type 1 (HSV-1) vector into a variety of sites in the mouse brain and assayed the regions of latency and expression of a β-glucuronidase (GUSB) cDNA from the latency-associated transcript promoter. Injection sites used were somatosensory cortex, visual cortex, striatum, dorsal hippocampus, and CSF spaces. Latent vector was detected in regions at a distance from the respective injection sites, consistent with axonal transport of vector. Regions of GUSB activity varied by injection site and included cerebral cortex, striatum, thalamus, hypothalamus, substantia nigra, hippocampus, midbrain, pons, medulla, cerebellum, and spinal cord. After a single injection, GUSB enzymatic activity reached wild-type levels in several brain regions. GUSB was found in some areas without any detectable vector, indicative of axonal transport of GUSB enzyme. GUSB-deficient mice, which have the lysosomal storage disease mucopolysaccharidosis (MPS) VII, have lysosomal storage lesions in cells throughout the brain. Adult MPS VII mice treated by injection of vector into a single site on each side of the brain had correction of storage lesions in a large volume of brain. The potential for long-term, widespread correction of lysosomal storage diseases with HSV-1 vectors is discussed.

Key Words: HSV-1, gene therapy, mucopolysaccharidosis VII, β-glucuronidase, lysosomal storage diseases, axonal transport

INTRODUCTION

Mucopolysaccharidosis (MPS) VII is caused by a single gene deficiency in the lysosomal enzyme β-glucuronidase (GUSB) and results in an inability to metabolize glycosaminoglycans (GAGs) [1]. Storage of undegraded GAGs affects a number of organ systems, including the liver, spleen, skeletal system, eye, heart, and brain (reviewed in [2]). MPS VII is rare in the human population, but over 40 lysosomal storage diseases (LSDs) have been identified and the collective body of LSDs has an occurrence of 1 in 7700 [3]. To study gene transfer to the brain, we have used the MPS VII mouse (reviewed in [4]) as a model LSD.

Lysosomal acid hydrolases (such as GUSB) are secreted from producing cells and taken up by neighboring cells in a process mediated by the mannose 6-phosphate receptor [5]. This secretion/uptake mechanism (referred to as cross-correction) results in a reversal of lysosomal storage in the vicinity of GUSB-producing cells [6,7]. GUSB has also been shown to be redistributed by axonal transport to distant sites [8,9]. Because of these two properties, correction of lysosomal storage can be achieved by expression of GUSB from a limited number of cells.

Abbreviations used: GUSB, β-glucuronidase; ISH, *in situ* hybridization; LAT, latency-associated transcript; HSV-1, herpes simplex virus type 1; MPS VII, mucopolysaccharidosis type VII.

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Several treatment options have been explored for MPS VII. All rely upon cross-correction. Treatments examined to date include tissue and cell transplants, enzyme replacement therapy, and gene therapy; correction of disease has been reported with each of these treatments in animal models (reviewed in [10]). However, correction of the adult brain has been problematic when using systemic treatments due to the inability of GUSB to cross the blood–brain barrier. Thus, gene therapy vectors that produce GUSB from within the brain are being explored.

A widespread distribution of GUSB activity is necessary to correct lysosomal storage, which is present in many regions of the MPS VII mouse brain [11]. A variety of GUSB-expressing viral vectors have been tested, and correction of lysosomal storage has been demonstrated in the MPS VII mouse brain for adeno-associated virus (AAV), adenovirus, and lentivirus (reviewed in [10,12,13]). However, these vectors show relatively limited spread from the injection site as tested by ISH or PCR and reversal of storage in more distant regions was attributed to cross-correction or axonal transport of GUSB activity. Injection of large animal brains has shown that nonneurotropic viral vectors (e.g., AAV, adenovirus, and lentivirus) efficiently transduce only the region around the needle track (similar to the results seen in mice). Examples include AAV in the cat [14] and adenovirus and lentivirus in the monkey [15,16]. It was estimated that a large brain would require hundreds of well-spaced injections to achieve total brain correction (e.g., AAV) [14]. Thus, a vector that is capable of a widespread transduction after a single injection holds much promise for the treatment of global disorders in large brains.

Herpes simplex virus type 1 (HSV-1) forms a latent infection in neurons that lasts for the lifetime of the infected individual (reviewed in [17,18]). Latency is characterized by undetectable levels of viral protein production and a gene expression profile limited to the latency-associated transcripts (LATs) [19–21]. The ability of the LAT gene to remain transcriptionally active in neurons has led to interest in using the virus as a gene therapy vector for the nervous system; preclinical data have shown efficacy in animal models of brain tumors, spinal root trauma, Parkinson disease, peripheral sensory neuropathy, and chronic pain syndromes (reviewed in [22]).

Alphaherpesviruses, of which HSV-1 is an example, use axonal transport to spread in the nervous system (reviewed in [23]). HSV-1 gene transfer vectors are able to spread from the injection site, with transduction of distant regions commonly reported [24–27], unlike the nonneurotropic viral vectors. A variety of injection sites have been examined in the rodent brain for transduction by HSV-1 vectors, including striatum [24–28], thalamus [25], medial forebrain bundle [29], hippocampus [24,26], cerebellar cortex [26], medial septum [24], and substantia nigra [24]. When a neuroattenuated HSV-1 strain was injected into the dog brain, latently infected cells were detected in the contralateral hemisphere by ISH [30]. Detection of positive cells in the contralateral hemisphere represents a substantial distance from the injection site in the dog brain (at least 10–30 mm) and indicates that HSV-1 has the ability to spread even greater distances when introduced into a larger brain. Spread of a gene transfer vector to distant brain regions has the potential to lead to a broad anatomical distribution of transgene expression, which will likely correlate with a larger sphere of disease correction.

The goal of this study was to determine the anatomical regions of both virus latency and the secreted transgene product (GUSB) following injection of a GUSB-expressing HSV-1 vector (1716-LAT-hGUSB) into a variety of anatomical sites in the mouse brain. We report that the neuroattenuated HSV-1 vector used is able to transduce multiple brain regions after a single injection. Wild-type levels of GUSB enzymatic activity were detected in a number of brain regions. We also show that the characteristic lysosomal storage pathology is corrected in the adult MPS VII mouse brain following intracranial injection with 1716-LAT-hGUSB, representing the first report of correction of an inherited disorder by HSV-1 gene therapy.

RESULTS AND DISCUSSION

Positive Cells Are Present in Specific Patterns After Vector Is Injected into Specific Sites

To evaluate the distribution of vector from various injection sites, we made unilateral injections into the right hemisphere with a titer of 3.4×10^5 pfu (in 1 μl) of either 1716 (empty vector) or 1716-LAT-hGUSB [27]. We injected C3H mice into the somatosensory cortex, visual cortex, striatum, and dorsal hippocampus (Fig. 1). We also made injections of 1 and 10 μl into the lateral ventricle and cisterna magna, respectively ($n = 4$). We removed the brains and analyzed them 2 months later. We used a histochemical stain [4] to determine the sites of GUSB activity (Fig. 2) after heat inactivation of the murine GUSB activity as shown previously [27]. Injection with the control vector resulted in undetectable GUSB staining (Fig. 2L). Injection with 1716-LAT-hGUSB resulted in abundant GUSB activity, which varied both by brain region and by relative amount of staining depending upon the site of vector injection (Figs. 2A–2K).

We counted GUSB-positive cells by brain region after injection into different sites (Table 1). Not all injection sites resulted in equal levels of staining. Injection into the striatum, somatosensory cortex, lateral ventricle, or

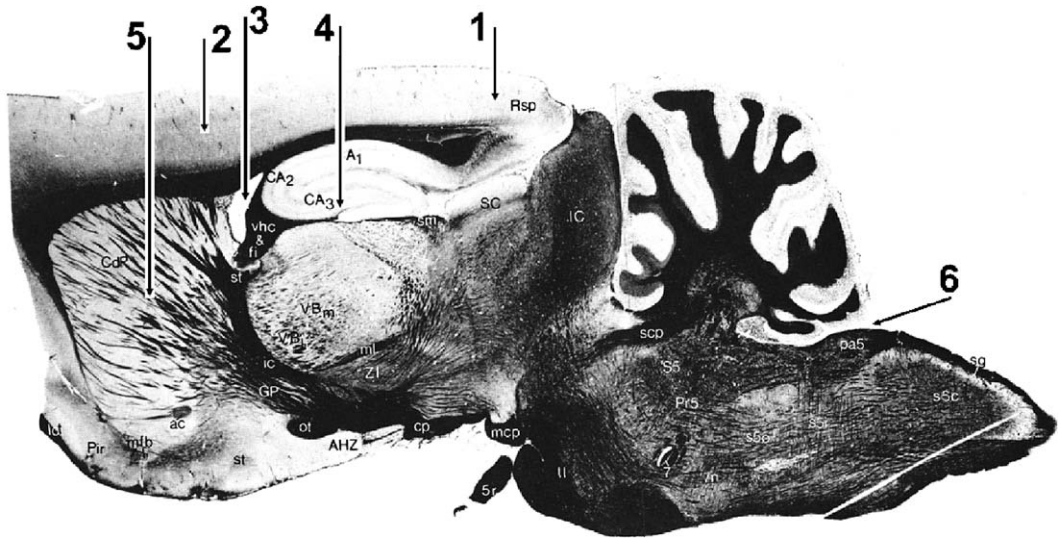

FIG. 1. Injection sites used in this study. A sagittal brain section illustrates the regions injected. All injections in the normal mouse were performed unilaterally into the right hemisphere. MPS VII mice were injected bilaterally into the somatosensory cortex. See Materials and Methods for precise coordinates. Key: (1) V1B region of visual cortex, (2) S1FL region of somatosensory cortex, (3) lateral ventricle, (4) CA3 layer of dorsal hippocampus, (5) striatum, (6) cisterna magna. Adapted, by permission of the publisher, from [50].

cisterna magna resulted in many positive cells and a widespread distribution of GUSB activity throughout the brain. With these injection sites, GUSB-positive cells could be found in virtually every coronal section from the frontal cortex to the caudal medulla. Injection into the visual cortex and dorsal hippocampus resulted in the fewest positive cells; however, the cells were still found in distant regions.

Certain brain regions did not exhibit GUSB-positive cells after injection into any of the parenchymal sites, while other regions were consistently positive regardless of the injection site. The hippocampus had few GUSB-positive cells (Fig. 2K), the cerebellum rare positive cells, and the olfactory bulbs no positive cells (data not shown). While many regions were consistently positive (including the thalamus, hypothalamus, midbrain, and dorsal regions of the pons and medulla), the patterns of specific neuronal nuclei within these regions differed by injection site. Some brain regions were effectively transduced by only a single injection site. For example, only injection into the somatosensory cortex led to robust GUSB activity in the ipsilateral striatum and ipsilateral substantia nigra. The spinal cord was positive only following injection into the cisterna magna.

Significant Levels of GUSB Enzymatic Activity Are Detected in Distal Brain Regions

Brains were divided into pieces and tissues were homogenized for a GUSB enzymatic activity assay (Table 2). Injection of 1716-LAT-hGUSB ($n \geq 10$) resulted in a significant increase in GUSB enzymatic activity above background in most of the brain regions. In some cases, wild-type levels of GUSB (13.8 nmol/h/mg in C3H whole brain) [27] were achieved, although it should be noted that GUSB activity is normally low in the C3H mouse compared to other strains [31]. Importantly, significant levels of GUSB were consistently detected in brain regions that were far from the injection site. We also measured GUSB activity in the thalamus (3.5 ± 0.78), midbrain (1.9 ± 0.28), and total brain (3.2 ± 1.0) following cisterna magna injection. The thalamus (Table 2, column E) and hypothalamus (column F) contained wild-type levels of GUSB activity regardless of the injection route, while the cerebral cortex (columns C and D) rarely contained significant levels of activity. Regions positive for the GUSB histochemical stain (Fig. 2) were generally also positive for GUSB enzymatic activity (Table 2).

LAT-Expressing Cells Are Also Widespread

We performed *in situ* hybridization with an antisense probe specific for the HSV-1 LATs to localize cells transduced directly by the vector (Fig. 3), in light of GUSB secretion. A sense riboprobe to the LAT sequence did not hybridize to latently infected tissues, and we detected no antisense hybridization in uninfected tissues. The LAT ISH signal was predominantly nuclear, as has been reported previously [32], and the signal was restricted to cells with the morphology of neurons. We counted LAT-positive cells by brain region (Table 1). We assayed the regions of LAT-positive cells by injection site (Table 3). The number of LAT-positive cells was similar to that seen by GUSB histochemistry

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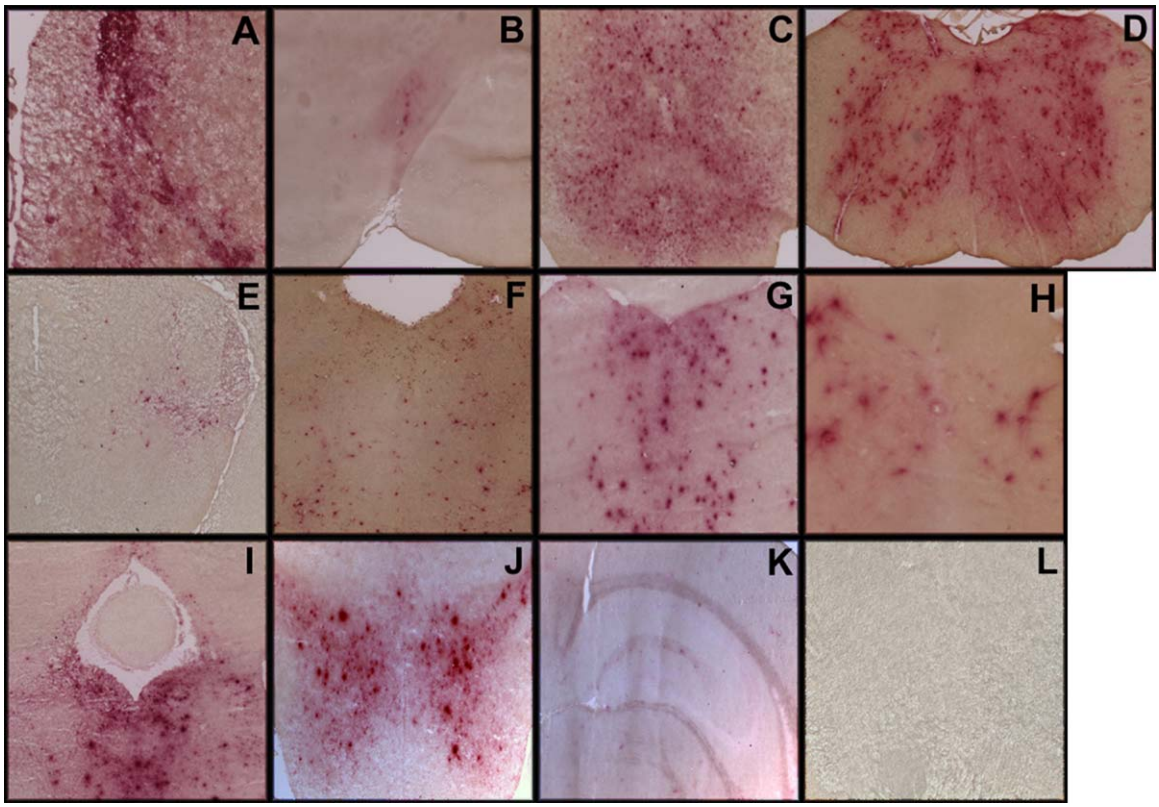


FIG. 2. Regions of GUSB expression after single, unilateral injection into different brain sites. 20-μm frozen sections were stained with naphthol-AS-B1-β-D-glucuronide. Shown are various examples of GUSB-positive regions after injection with 1716-LAT-hGUSB (with injection site indicated): (A) ipsilateral striatum (somatosensory cortex), (B) ipsilateral substantia nigra (somatosensory cortex), (C) midbrain (striatum), (D) medulla (cisterna magna), (E) ipsilateral caudal thalamus (visual cortex), (F) pons (cisterna magna), (G) rostral medulla (lateral ventricle), (H) thoracic spinal cord (cisterna magna), (I) pontine/mesencephalic boundary (lateral ventricle), (J) hypothalamus (striatum), (K) hippocampus (dorsal hippocampus), (L) ventral midbrain (lateral ventricle injection with 1716). Original magnification 40×.

(Table 1) for most brain regions. Interestingly, the cerebral cortex and hippocampus contained many LAT-positive cells for certain injection sites, but few GUSB-positive cells.

To determine if axonal transport of the vector could explain the transduction patterns found in this study, we examined neural pathways to document the existence of pathways linking regions of latency to the injection sites. We studied two injection sites (striatum and somatosensory cortex) in detail. Of the 17 neuronal nuclei that consistently harbored latent virus after striatal injection, direct neural pathways to the injection site were documented for 11 of these regions (65%). Of the 11 nuclei that harbored latent virus after somatosensory cortex injection, neural pathways existed for 9 of these regions (82%).

Evidence for Axonal Transport of GUSB Activity

We found GUSB-positive cells in the ipsilateral substantia nigra (SN) (Fig. 2B) after somatosensory cortex injection (4/4 animals), but no LAT-positive cells in the SN or any adjacent region (0/4 animals, Fig. 3G). The SN sends axonal projections to the striatum and abundant GUSB activity was present in the striatum (Fig. 2A), indicating that GUSB moved by retrograde axonal transport from the striatum to the SN. Axonal transport of GUSB has previously been shown following transduction by AAV vectors [8,33].

To determine if the vector was present in the SN, we performed quantitative PCR on DNA isolated from tissue sections of the ipsilateral SN ($n = 4$) to detect the vector genome. The contralateral SN did not contain GUSB-expressing cells, and tissues from this region served as a negative control. The somatosensory cortex did contain LAT-positive cells after striatal injection (by ISH), and tissues from this region served as a positive control for a known site of vector genomes. All four positive controls contained detectable genome sequences (0.07 ± 0.03 genomes per cell), while no genomes were detected in either the negative control or the ipsilateral substantia nigra. Thus, the vector genome was not transported to this region. We took the inability to detect vector

TABLE 1: Mean GUSB-positive and LAT-positive cells per brain region

Injection site	Brain regions assayed for GUSB histochemical stain and LAT *in situ* hybridization															
	Cortex		Striatum		Diencephalon		Hippo-campus		Substantia nigra		Midbrain/pons/medulla		Cerebellum		Spinal cord	
	GUS[a]	ISH[b]	GUS	ISH	GUS	ISH	GUS	ISH	GUS	ISH	GUS	ISH	GUS	ISH	GUS	ISH
Somatosensory cortex	+	+	+++	++	+++	+++	–	–	++	–	++	++	–	+	nd	nd
Visual cortex	+	+	+	+	++	++	+	+	–	–	+	++	–	+	nd	nd
Striatum	+	+++	–	+	++++	+++	–	++	–	–	++++	+++	+	–	nd	nd
Dorsal hippocampus	+	+	+	–	++	++	+	+	+	–	+	++	+	+	nd	nd
Lateral ventricle	++	++++	+	–	++++	+++	+	+	–	–	++++	+++	+	–	–	nd
Cisterna magna	–	nd	–	nd	++++	nd	–	nd	–	nd	++++	nd	+	nd	++	nd

Brain sections (n = 4 animals and 20 sections) were stained for GUSB histochemical activity or probed for LAT expression by ISH. Key: –, no positive cells on any section at this region; +, average of 1–5 positive cells per section in this region; ++, 5–15; +++, 15–50; ++++, more than 50; nd, not determined.

[a] GUSB histochemical stain.

[b] *In situ* hybridization (ISH) toward LATs. ISH was not performed after injection into the cisterna magna.

genome in the SN, combined with known axonal pathways to a region expressing high levels of GUSB, as evidence that GUSB moved via axonal transport to the SN.

Normal and MPS VII Mice Express GUSB Histochemical Activity in Similar Regions

To determine if the 1716-LAT-hGUSB vector was capable of reversing the lysosomal storage associated with GUSB deficiency, we injected MPS VII mice bilaterally in the somatosensory cortex (n = 3). The 1716-LAT-hGUSB vector expresses human GUSB, which has been shown to correct lysosomal storage in the MPS VII mouse brain [6,7,34]. After 2 months, we removed treated brains and cut them in half at the midline. We froze the left hemisphere and sectioned it for histochemical staining.

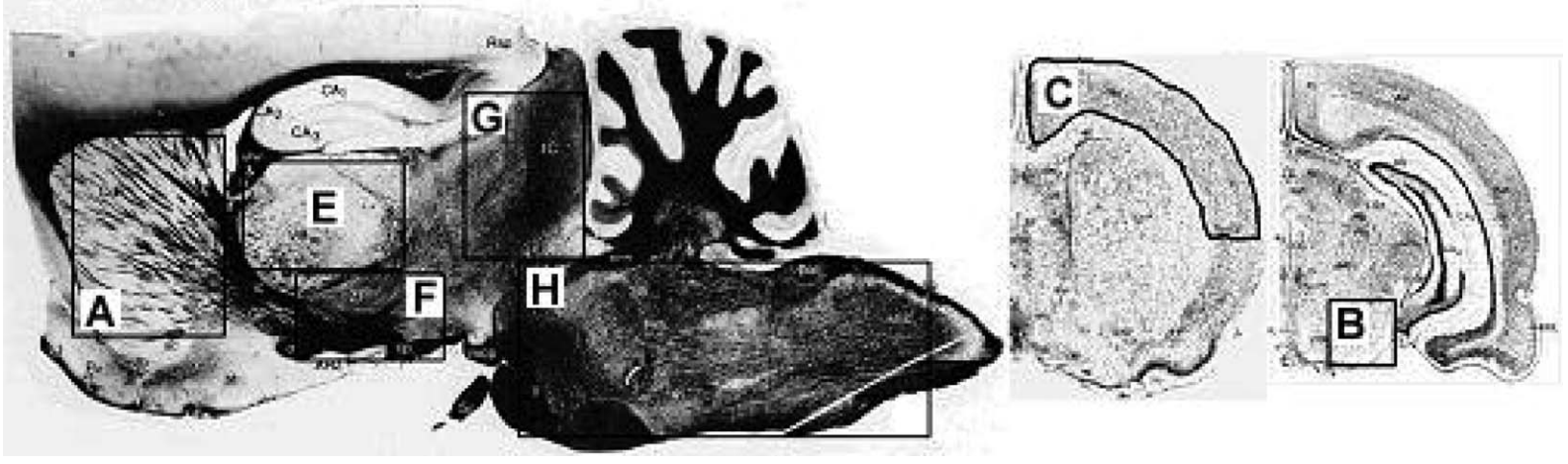
GUSB deficiency leads to an accumulation of glycosaminoglycans, including heparin sulfate proteoglycans (HSPs) [1]. As HSPs are involved in the initial binding event of HSV-1 entry [35], it is possible that tropism and virus spread differ between MPS VII mice and those unaffected by GUSB deficiency. However, we found that the regions of GUSB activity were similar in MPS VII mice (Fig. 4) and normal mice (Fig. 2). We detected many GUSB-positive cells in the striatum, thalamus, hypothalamus, and medulla. As was seen in unaffected mice, GUSB activity was not expressed in every region after somatosensory cortex injection (notably the olfactory bulb, cerebellum, hippocampus, and only rare positive cells in the cerebral cortex).

Lysosomal Storage is Reversed in a Large Volume of Brain

To evaluate the presence of lysosomal storage, we embedded the right hemisphere from each of three treated MPS VII mice in plastic for histological analysis (Fig. 5). Untreated adult MPS VII brains had abundant lysosomal storage in many brain regions, while the normal mouse brain did not exhibit storage in any region. Following vector injection, we detected no storage in the striatum, piriform cortex, thalamus, hypothalamus, zona incerta/substantia nigra, or preoptic area (storage was apparent in each of these regions in untreated controls). This correlated with the presence of GUSB histochemical activity in these regions in the other hemisphere. Storage was still evident in the olfactory bulb, cerebellum, hippocampus, and several cortical regions after treatment; however, these regions had little to no GUSB histochemical activity in the other hemisphere. We detected GUSB activity additionally in the midbrain, pons, and medulla, but vacuolar storage lesions are not prominent in these regions in the MPS VII mouse.

TABLE 2: GUSB enzymatic activity in various brain regions following vector inoculation into different sites

	Regions assayed for GUSB enzymatic activity															
	A		B		C		D		E		F		G		H	
Injection site	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]	Units[a]	[b]
Somatosensory cortex	11.7 ± 3.2*	5.0	17.8 ± 5.5*	5.3	0.24 ± 0.1	1.0	1.0 ± 0.1	1.7	13.2 ± 3.8*	4.2	13.2 ± 4.6*	3.8	6.7 ± 3.0	3.0	11.8 ± 1.3*	3.2
Visual cortex	0.3 ± 0.1	1.2	3.4 ± 0.6*	1.8	1.3 ± 0.1	1.1	2.1 ± 0.2*	1.5	17.7 ± 4.0*	7.2	37.9 ± 8.9*	10	20.6 ± 4.5*	9.1	0.9 ± 0.2	1.4
Striatum	3.7 ± 0.2*	1.7	1.8 ± 0.2	1.4	1.4 ± 0.6	1.6	1.5 ± 0.9	1.3	20.1 ± 6.1*	7.1	36.7 ± 9.2*	17	nd	nd	7.9 ± 2.0*	18
Dorsal hippocampus	0.9 ± 0.1	1.1	4.0 ± 0.4*	1.7	0.42 ± 0.1	1.4	5.3 ± 0.7*	1.9	17.4 ± 6.4*	5.9	17.8 ± 6.2*	4.7	6.3 ± 2.5*	3.2	0.9 ± 0.3	1.2
Lateral ventricle	8.0 ± 1.9*	2.2	10.6 ± 1.8*	3.3	2.3 ± 1.6	2.3	1.2 ± 1.1	1.8	13.8 ± 2.1*	8.4	23.7 ± 3.9*	13	13.9 ± 2.9*	23	11.3 ± 3.3*	6.5



GUSB enzymatic activity (units expressed in nmol/h/mg) was measured in brain homogenates by enzymatic activity assay ($n = 10–14$). The brain pieces used in the assay are shown in the schematic above and contain the following major structures: A, striatum; B, substantia nigra; C, ipsilateral cortex; D, contralateral cortex (same as C but from other hemisphere); E, thalamus; F, hypothalamus; G, midbrain; H, pons/medulla. GUSB activity is reported in two ways: [a]units of activity (nmol/h/mg) after correction for background activity detected in 1716-injected animals and [b]fold expression above background. Standard error is shown. nd, not determined.

* $P < 0.05$ for paired t test. Figure adapted, by permission of the publisher, from [50].

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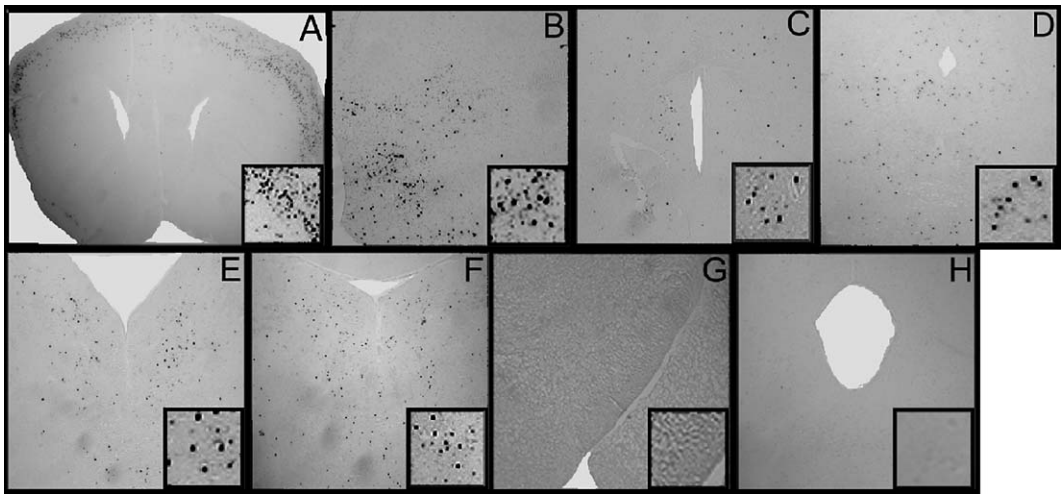

FIG. 3. Regions of LAT expression after unilateral injection into different brain sites. Shown are various examples of LAT-expressing regions after injection with 1716-LAT-hGUSB (with injection site indicated): (A) rostral cortex (striatum), (B) preoptic area (lateral ventricle), (C) rostral midbrain (striatum), (D) caudal midbrain (somatosensory cortex), (E) pons (dorsal hippocampus), (F) medulla (visual cortex), (G) substantia nigra (somatosensory cortex), (H) uninjected. Original magnification 40× (10× for A).

In this study, we have shown that a neuroattenuated HSV-1 vector is able to establish latency (Fig. 3) and express GUSB (Fig. 2) in several brain regions at a distance from the intracranial injection site. We found wild-type levels of GUSB enzymatic activity in several brain regions after a single injection. Interestingly, following a single, unilateral injection, equal transduction between the two hemispheres was noted in almost every positive region (Fig. 2). Transduction spanned nearly the entire brain along the rostral/caudal axis, with LAT-expressing cells detected from the frontal cortex to the caudal medulla after striatal injection (a total distance of 10 mm). Correction of lysosomal storage was demonstrated in several brain regions, some of which were at a distance from the injection site (Fig. 5).

Axonal transport has been proposed to be the primary method of movement of attenuated HSV-1 vectors [24–26]. However, direct demonstration of axonal transport requires nanoliter injections, which have not been reported to date with attenuated HSV gene transfer vectors. The widespread distribution of latently infected cells in this study is not likely to be due to diffusion of vector, as injection of the same volume (1 μl) with other viral vectors has resulted in a maximal transduction of 1–2 mm from the needle track [7,8,36–40]. It should be noted that some nonneurotropic viral vectors have been shown to perform axonal transport, including AAV, adenovirus [41], and lentivirus pseudotyped with rabies glycoprotein [42]. However, the efficiency of axonal transport with these vectors relative to HSV-1 (which is obligated to use axonal transport during the life cycle) has not been determined.

The transduction patterns produced by HSV-1 vectors differ compared to those afforded by nonneurotropic vectors. Specifically, other GUSB-expressing viral vectors transduce almost every cell at the injection site and these cells produce large amounts of GUSB [7,8]. Our HSV-1 vector transduced relatively few cells in any given region, but it was able to transduce many different regions after a single injection (Fig. 3). Although the GUSB activity produced was insufficient to provide histochemical activity in every cell (Fig. 2), it was sufficient to reverse the pathology of the disease in broad regions (Fig. 5). This finding was likely due to the secretory properties of GUSB. Although cross-correction results in significant correction in the small brain of a mouse after intracranial injection, the total distance of GUSB activity from the needle track was similar in the much larger cat brain [14]. Thus, to achieve significant levels of cross-correction in a larger brain it will be necessary to transduce cells in many brain regions. Further analysis of transduction by HSV-1 vectors in the large animal brain is needed to determine if the transduction patterns found in the mouse in this study will match the homologous regions in a larger brain.

Most latent regions could be attributed to axonal transport of vector from the injection site, due to the existence of neural pathways linking these sites (Table 3). An examination of the directionality of axonal transport revealed that the vector was able to move in both the retrograde and the anterograde directions, although most regions of LAT-expressing cells were consistent with retrograde transport. For example, after somatosensory cortex injection, LAT-expressing cells

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TABLE 3: Regions of LAT-positive cells following injection into different brain regions

	Regions positive for LAT ISH				
Injection site	Forebrain	Cerebral cortex	Diencephalon	Midbrain	Pons/medulla
Somatosensory cortex	Caudate nucleus	Frontal association, orbital, prelimbic	Ventral posterolateral thalamic nucleus	Periaqueductal gray, mesencephalic nucleus, raphe nucleus	Gigantocellular reticular nucleus, solitary tract nucleus
Visual cortex	None	None	Preoptic nucleus	Periaqueductal gray	Gigantocellular reticular nucleus, solitary tract nucleus, intercalated nucleus of the medulla
Striatum	None	Cingulate, motor, somatosensory, insular, piriform, auditory	Preoptic nucleus, medullary reticular nucleus	Raphe nuclei, periaqueductal gray, reticular nuclei, intermediate nucleus lateral lemniscus, tectospinal tract, external cortex of the inferior colliculus, superior colliculus	Cuneate nucleus, solitary tract nucleus, prepositus nucleus, intercalated nucleus of the medulla
Dorsal hippocampus	None	Somatosensory, motor, piriform	Nucleus of the posterior commissure, intermediate reticular nucleus, superior colliculus	Periaqueductal gray	Solitary tract nucleus, medial vestibular nucleus, hypoglossal nucleus, gigantocellular reticular nucleus
Lateral ventricle	Caudate nucleus	Cingulate, motor, somatosensory, insular, piriform, auditory	Preoptic nucleus, intermediate reticular nucleus	Periaqueductal gray	Solitary tract nucleus, gigantocellular reticular nucleus

LAT-positive cells were localized by ISH (n = 4). At least 3/4 animals were positive in each of these regions.

were detected in the ventral posterolateral thalamic nucleus (indicative of retrograde transport) and the striatum (indicative of anterograde transport) (neural pathways reviewed in [43]). Interestingly, to achieve gene expression after anterograde axonal transport, the vector would have to cross a synapse and enter another cell body. Thus, the data indicate that neuroattenuated HSV-1 vectors are able to traverse at least one neuronal synapse and achieve gene expression in a secondary neuron.

With few exceptions, regions positive for GUSB histochemical stain (Fig. 2) were also positive for gene expression (Fig. 3). However, GUSB activity was detected in the ipsilateral SN after somatosensory cortex injection by both histochemistry (Fig. 2B) and enzyme assay (Table 2), but neither gene expression (Fig. 3G) nor vector genomes were detected in this region, indicating that the vector was not transported to the SN. Injection into the somatosensory cortex did result in many positive cells in the ipsilateral striatum (Fig. 2A), which receives projections from the SN (reviewed in [44]). Evidence for axonal transport of GUSB to distant regions has been shown previously [8,9]. Thus, we conclude that GUSB activity moved from the striatum to the SN via retrograde axonal transport. This represents the first demonstration of axonal transport of GUSB following transduction by a HSV-1 vector. Transport of GUSB to distant regions expands the distribution of activity, which is important for an effective treatment of a widespread disorder like MPS VII.

Some brain regions were found to contain many LAT-expressing cells, but little or no GUSB activity (Tables 1 and 2). The most prominent example was the neocortex after striatal injection (Fig. 3A). Thus, although the GUSB cDNA was inserted into the 5′ end of the LAT gene [27], expression of LATs did not always correlate with production of detectable GUSB activity. This finding suggests that there may be posttranscriptional regulation of LAT gene-mediated transgene expression in some cells, as has been proposed previously [45].

Abundant GUSB activity was detected in additional brain regions (midbrain, pons, and medulla) after treatment of MPS VII mice. However, vacuolar storage lesions were not common in these regions in untreated MPS VII mice; thus these regions could not be evaluated

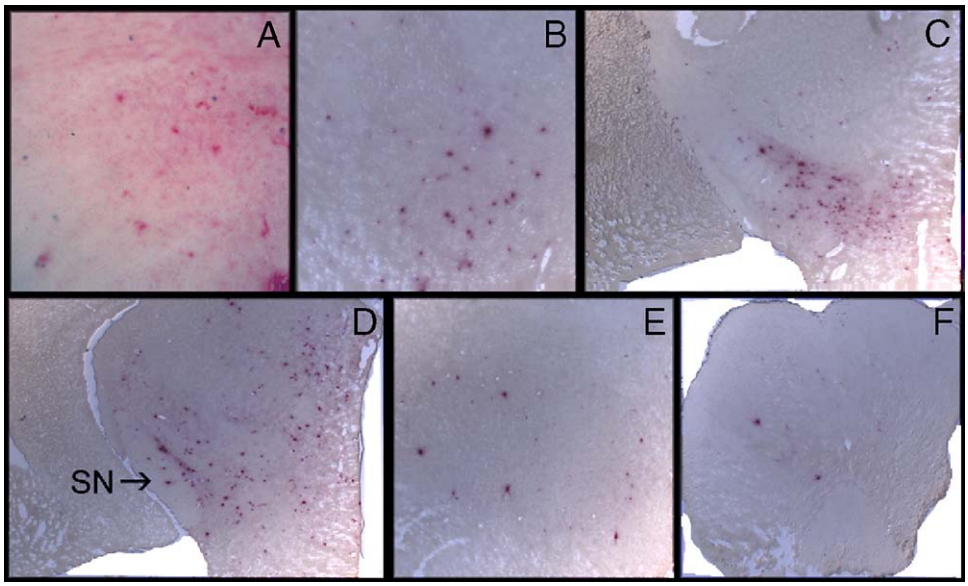

FIG. 4. GUSB histochemical stain in the MPS VII brain after treatment with 1716-LAT-hGUSB. 20-μm frozen sections from the left hemisphere were stained with naphthol-AS-B1-β-D-glucuronide. Positive staining was evident in many brain regions. Representative sections are shown: (A) frontal cortex, (B) preoptic area, (C) rostral midbrain, (D) caudal midbrain, (E) pons, (F) medulla. Untreated MPS VII brains do not exhibit any staining (data not shown), similar to control brains after heat treatment (see Fig. 2L). Original magnification 40× (10× for C and D).

for correction of the storage pathology. Lysosomal storage does occur in these regions in other LSDs (e.g., α-mannosidosis) [46]. Thus, transduction of these regions is potentially beneficial for correction of other LSDs.

This study represents the first demonstration of correction of an inherited disorder with HSV-1 gene therapy. Furthermore, the vector was shown to correct multiple brain regions following injection into a single site. We have previously shown that the 1716-LAT-hGUSB vector is capable of stable expression (up to 1 year postinjection) at levels higher than the therapeutic range (1–5% of normal) [27]. Thus, this vector has the potential for long-term correction of a significant fraction of the brain.

MATERIALS AND METHODS

Cells and viruses. Vero cells were grown in Dulbecco's modified Eagle's medium (Gibco BRL, Gaithersburg, MD, USA) containing 5% fetal calf serum, 100 μg/ml penicillin, and 100 μg/ml streptomycin. The 1716-LAT-hGUSB vector was described previously [27]. Vector was both grown and titered (by plaque assay) on Vero cells, as previously described [20], to a titer of 3.4×10^8 pfu/ml.

Intracranial injections. This study was approved by the Institutional Animal Care and Use Committee and the *Principles of Care and Use of Laboratory Animals* of the NIH was followed. Four- to 6-week-female C3H HeJ mice from The Jackson Laboratory (Bar Harbor, ME, USA) were anesthetized using a mixture of ketamines/xylazines. Mice were inoculated intracranially with 3.4×10^5 pfu (in 1 μl) into the right hemisphere using a small-animal stereotactic apparatus (Kopf Instruments; Tujunga, CA, USA) as described previously [27]. *The Mouse Brain in Stereotaxic Coordinates* [47] was used to identify correct brain coordinates for the various injection sites. Coordinate dimensions used are described as [(1) distance to right of midline, (2) distance caudal of bregma, and (3) depth from pial surface] somatosensory cortex, 2.50, 0.50, 1.00 mm; visual cortex, 2.75, 3.50, 1.00 mm; striatum, 2.00, 0.00, 3.00 mm; dorsal hippocampus, 1.50, 2.00, 2.00 mm; lateral ventricle, 1.00, 0.20, 2.0 mm. Virus was injected using a 10-μl Hamilton syringe with a 26s-gauge needle. After injection the needle remained in place for 1 min and then was slowly withdrawn over 30 s. Injections into the cisterna magna were of a total volume of 10 μl, but the same total titer (3.4×10^5 pfu). Six- to 8-week-old MPS VII mice (C3H/HeOuJ-*Gus*$^{mps\text{-}2J}$) were anesthetized and injected bilaterally (1 μl each) into the somatosensory cortex as detailed above.

Tissue preparation. Mice were sacrificed 2 months postinjection as follows: animals were anesthetized and then transcardially perfused with 10 ml cold 1× PBS (DEPC) followed by 10 ml 4% paraformaldehyde in 1× PBS (DEPC). Brains for sectioning were removed, postfixed in 4% paraformaldehyde, and cryoprotected by sinking in 10% and then 30% sucrose (DEPC). Brains were then frozen in OCT (Tissue-Tek, Torrance, CA, USA) over a bed of dry ice and sectioned to 20 μm. Brains for GUSB quantitation by enzyme assay were removed and immediately frozen. MPS VII mouse brains were cut down the midline, and the left hemisphere was frozen and sectioned to 20 μm. The right hemisphere was embedded in JB-4 Plus medium (Polysciences; Warrington, PA, USA) and sectioned to 1 μm and then stained with toluidine blue.

In situ GUSB histochemical stain. Regions of GUSB activity were localized in frozen tissue sections using naphthol-AS-B1-β-D-glucuronide (Sigma) as substrate [4]. Murine GUSB activity was heat inactivated as described previously [27]. Four brains were sectioned coronally and at least 20 sections (representing a cross section of the entire brain) were stained from each brain.

In situ hybridization for LAT gene expression. An adaptation of a protocol previously described [48] was used to perform *in situ* hybridization. DIG-labeled antisense and sense riboprobes for detection of LATs were generated as described previously [27]. Four brains were sectioned coronally and at least 20 sections were stained.

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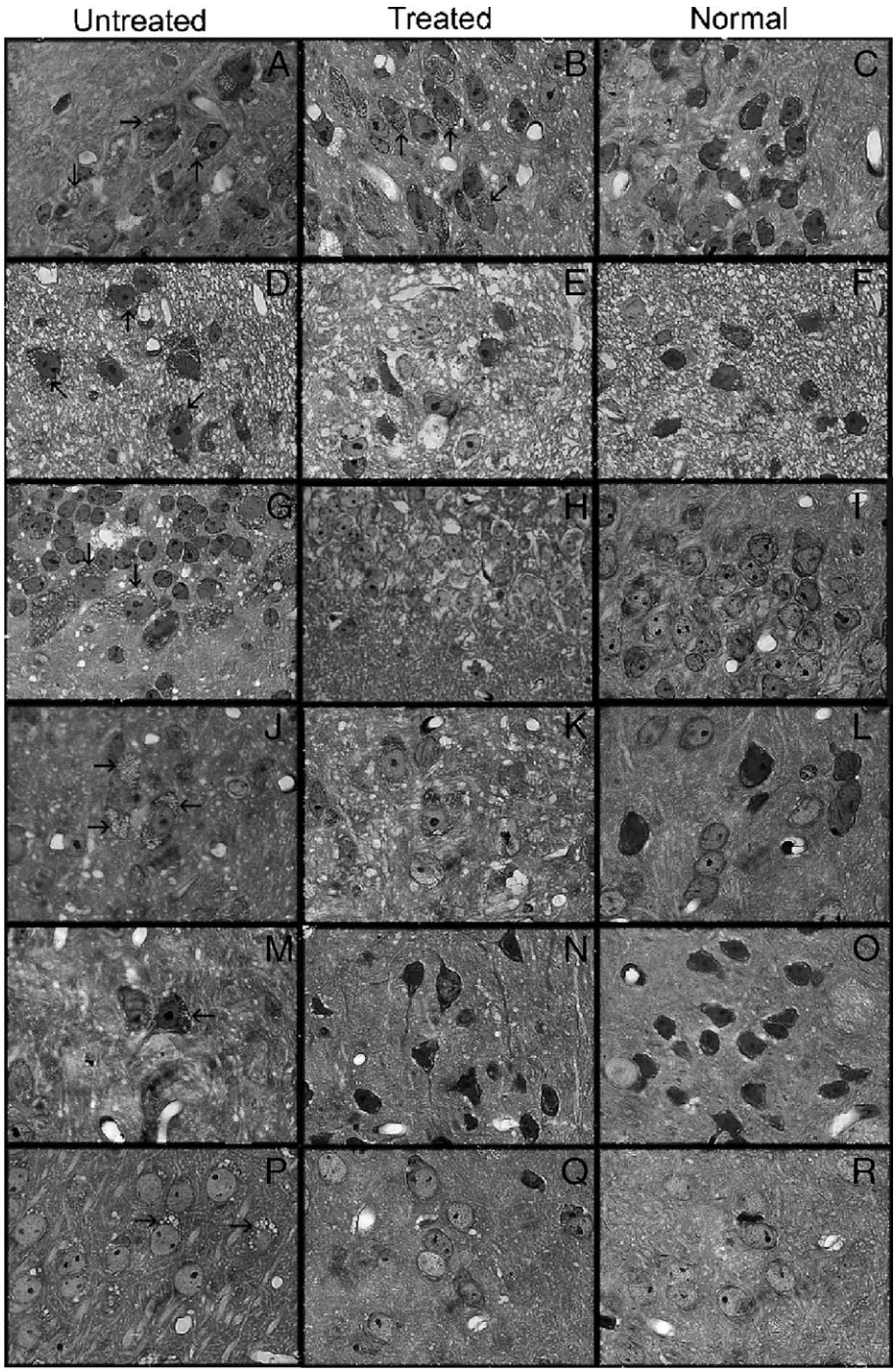

FIG. 5. Correction of lysosomal storage after treatment with 1716-LAT-hGUSB. (A, D, G, J, M, P) Lysosomal storage (indicated by arrows) is present in many regions in the adult MPS VII mouse brain. (B, E, H, K, N, Q) Lysosomal storage is corrected in many brain regions after treatment with 1716-LAT-hGUSB. (C, F, I, L, O, R) The normal mouse exhibits no lysosomal storage. Examples are shown from the olfactory bulb (A–C), striatum (D–F), piriform cortex (G–I), zona incerta (J–L), thalamus (M–O), and hypothalamus (P–R). Original magnification 600×.

Quantitation of GUSB activity. Brain pieces were dissected out using a scalpel under a dissection microscope. All tissues were first heat inactivated as outlined above. Assays were carried out using 4-methylumbelliferyl β-D-glucuronide (Sigma) as described previously [27].

PCR of vector DNA. Tissue from the region of the substantia nigra ($n = 4$) was scraped off of slides (approx 0.5 mg) and DNA was extracted as reported previously [49]. The primers and probe used for real-time PCR amplification were toward the thymidine kinase gene [49].

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