Prion Strain Targeting Independent of Strain-Specific Neuronal Tropism[▽]

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While neuropathological features that define prion strains include spongiform degeneration and deposition patterns of PrP^{Sc}, the underlying mechanism for the strain-specific differences in PrP^{Sc} targeting is not known. To investigate prion strain targeting, we inoculated hamsters in the sciatic nerve with either the hyper (HY) or drowsy (DY) strain of the transmissible mink encephalopathy (TME) agent. Both TME strains were initially retrogradely transported in the central nervous system (CNS) exclusively by four descending motor tracts. The locations of HY and DY PrP^{Sc} deposition were identical throughout the majority of the incubation period. However, differences in PrP^{Sc} deposition between these strains were observed upon development of clinical disease. The differences observed were unlikely to be due to strain-specific neuronal tropism, since comparison of PrP^{Sc} deposition patterns by different routes of infection indicated that all brain areas were susceptible to prion infection by both TME strains. These findings suggest that prion transport and differential susceptibility to prion infection are not solely responsible for prion strain targeting. The data suggest that differences in PrP^{Sc} distribution between strains during clinical disease are due to differences in the length of time that PrP^{Sc} has to spread in the CNS before the host succumbs to disease.

Prion diseases are fatal neurodegenerative disorders that affect animals, including humans. Prions are comprised of PrP^{Sc}, an abnormal isoform of the host-encoded prion protein, PrP^C, and lack a nucleic acid genome (6, 10, 26, 27). Prion strains are operationally defined by differences in the distribution of spongiform degeneration or PrP^{Sc} deposition within the central nervous system (CNS) following experimental passage (4, 11, 14, 15, 20). While there is evidence to suggest that prion strain diversity is encoded by the conformation of PrP^{Sc} (4, 7, 17, 28, 29), the mechanism(s) responsible for strain-specific differences in neuropathology is not known.

Prion strains were initially identified by the characteristic intensity of spongiform degeneration in different regions of the CNS (13, 14). It was subsequently demonstrated that the anatomical distribution of PrP^{Sc} deposition in the CNS differs between prion strains in rodents (4, 8, 15). It is not known if neuronal susceptibility to strain-specific prion infection contributes to prion strain targeting; however, more than one prion strain can infect a given brain nucleus (9).

Initial evidence for prion spread along specific neuroanatomical pathways was derived from experiments using intraocular inoculation. The results from that study indicated that the temporal and spatial detection of spongiform degeneration was consistent with prion transport along the retinotectal pathway (12). Subsequent studies with hamsters, employing a variety of peripheral prion infections, all came to the same conclusion: prions were preferentially transported along defined neuroanatomical pathways (1, 2, 24). While it is clear that PrP^{Sc} is selectively transported via neuroanatomical routes, it is not known if strain-specific transport underlies the charac-

teristic differences in the localization of PrP^{Sc} between prion strains.

To further investigate the mechanisms of strain-specific differences in distribution of PrP^{Sc} upon development of clinical disease, we inoculated hamsters in the sciatic nerve with either the hyper (HY) or drowsy (DY) strain of the transmissible mink encephalopathy (TME) agent and examined the spread of PrP^{Sc} from this common site of infection. We determined that both TME strains are transported via the same four descending motor tracts of the CNS, suggesting that strain-specific differences in axonal transport do not exclusively determine prion strain targeting. Additionally, we did not identify any populations of neurons that are resistant to TME infection, suggesting that strain-specific differences in neuronal susceptibility to prion infection do not fully explain strain targeting. One possible explanation for these results is that the strain-specific distribution of PrP^{Sc} upon development of clinical disease is determined by the length of time PrP^{Sc} transsynaptically spreads before the death of the host.

MATERIALS AND METHODS

Animal inoculations and tissue collection. All procedures involving animals were approved by the Creighton University Institutional Animal Care and Use Committee and were in compliance with the *Guide for the care and use of laboratory animals*. Male 10- to 11-week-old Syrian hamsters (Harlan-Sprague-Dawley, Indianapolis, IN) were used in these studies. Sciatic nerve inoculations were performed as previously described (3). Either one microliter of a 1% (wt/vol) brain homogenate containing 10^{5.2} intracerebral (i.c.) 50% lethal doses (LD₅₀)/ml of the HY TME agent or 10^{3.1} i.c. LD₅₀/ml of the DY TME agent or a mock-infected homogenate was injected. Other hamsters were i.c. inoculated with 25 μ l of a 1% (wt/vol) brain homogenate containing 10^{6.6} i.c. LD₅₀/ml of the HY TME agent. All animals were observed three times per week for the onset of neurological disease, with a clinical diagnosis of HY TME based on the presence of ataxia and hyperexcitability while clinical diagnosis of DY TME was based on the appearance of progressive lethargy (5). The incubation period was calculated as the number of days between inoculation and the onset of clinical signs. At selected time points postinfection, infected and mock-infected hamsters were anesthetized with isoflurane and perfused transcardially with 50 ml of 0.01 M Dulbecco's phosphate-buffered saline followed by 75 ml of McLean's paraformaldehyde-lysine-periodate (PLP) fixative. The brain, brainstem, dorsal root

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TABLE 1. PrP^{Sc} immunohistochemistry analysis of processed tissue sections

Tissue	Section thickness (μm)	No. of adjacent tissue sections per slide ^b	Sampling interval (μm) of tissue sections from ^a :		Avg no. of tissue sections analyzed per infected animal ^b
			Infected animals	Mock-infected animals	
DRG	7	15	0	0	225
Sympathetic ganglia	7	9	0	0	405
Spinal cord	7	4	196	420	76
DY brain	7	4 (caudal), 8 (rostral)	126	420	80 (caudal), 96 (rostral)
HY brain	7	4 (caudal), 8 (rostral)	126	420	40 (caudal), 40 (rostral)

^a Maximum distance between tissue sections.
^b Caudal tissue includes medulla, pons, and cerebellum; rostral tissue includes mesencephalon, diencephalon, and telencephalon.

ganglia (DRG), sympathetic chain with ganglia attached, and spinal cord were immediately removed and placed in PLP for 5 to 7 h at room temperature prior to paraffin processing. Injection of dextran into the sciatic nerve was performed as described previously to confirm that the correct levels of DRG, sympathetic ganglia, and lumbar spinal cord were collected (data not shown) (3).
PrP^{Sc} immunohistochemistry. PrP^{Sc} immunohistochemistry analysis was performed as described previously (23). Briefly, PLP-fixed, paraffin-embedded tissue sections were cut to a thickness of 7 μm and attached to glass slides (Fisher Scientific, Atlanta, GA). Following deparaffinization, the sections were incubated in 95% formic acid (Sigma-Aldrich, St. Louis, MO) for 20 min at room temperature. Endogenous peroxidases were blocked by immersion of sections in 0.3% H₂O₂ in methanol for 20 min at room temperature, and nonspecific staining was blocked by immersion in 10% normal horse serum (Vector Laboratories, Bur-

lingame, CA) in Tris-buffered saline for 30 min at room temperature. The sections were incubated with the monoclonal anti-PrP antibody 3F4 (Chemicon, Temecula, California) (18) at 4°C overnight. The sections were incubated with a biotinylated horse anti-mouse immunoglobulin G conjugate and subsequently incubated in ABC solution (Elite kit; Vector Laboratories, Burlingame, CA). Sections were developed using 0.05% (wt/vol) 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO) in Tris-buffered saline containing 0.0015% H₂O₂ and counterstained with hematoxylin (Richard Allen Scientific, Kalamazoo, MI). Light microscopy was performed using a Nikon i80 microscope (Nikon, Melville, NY), and images were captured as described in the previous section. PrP^{Sc} deposition was defined by a punctate pattern of immunoreactivity that was identified only in TME-infected animals. This deposition pattern differs from the weak diffuse staining that was also present in the mock-infected animals.

FIG. 1. PrP^{Sc} deposition in DRG, sympathetic ganglia, and lamina IX of the lumbar spinal cord following mock, DY, or HY TME agent inoculation of the sciatic nerve. In mock-infected hamsters, PrP^{Sc} was not detected in DRG (A), sympathetic ganglia (D), or VMNs in lamina IX (G). At 63 days post-DY TME inoculation, PrP^{Sc} was detected in neurons of the DRG (B), while PrP^{Sc} was not detected in either the neurons of the sympathetic chain at 60 days p.i. (E) or laminae IX of the lumbar spinal cord at 63 days p.i. (H). In HY TME-inoculated animals at 7 days p.i., PrP^{Sc} was detected in DRG neurons (C) while PrP^{Sc} was not detected in neurons of the sympathetic chain (F) or VMNs of the lumbar spinal cord (I). Scale bar, 50 μm.

TABLE 2. Temporal and spatial distribution of PrP^{Sc} in brain and brainstem following sciatic nerve inoculation with DY TME agent

CNS region	PrP ^{Sc} immunostaining at indicated no. of days postinfection ^a				
	77	84	111	127	139
Medulla - Pons					
Reticular formation	0	+ ^d	++	+++	++++
Lateral vestibular nucleus	0	0	+ ^b	++	++++
Cerebellum					
Interposed nucleus	0	0	+ ^b	++	+++
Mesencephalon					
Red nucleus	ND	0	0	++	++++
Diencephalon					
Reticular thalamic nucleus	ND	0	0	+ ^{c,d}	+++
Ventroposterior thalamic nucleus	ND	0	0	+ ^{c,d}	++
Telencephalon					
Hind limb cortex	ND	0	0	++	+++

^a Relative intensities of PrP^{Sc} immunostaining: 0, none; +, rare; ++, weak; +++, moderate; +++++, heavy. ND, not done.

^b Asymmetrical staining pattern ipsilateral to inoculation site.

^c Asymmetrical staining pattern contralateral to inoculation site.

^d PrP^{Sc} was detected in one of three animals examined.

The sampling frequency of the processed tissues is shown in Table 1 to define the parameters used to determine the detection of PrP^{Sc} in each structure at the various time points. At each time point postinfection, three infected animals and two mock-infected animals were examined. For HY and DY TME agent-infected hamsters, tissue sections were analyzed at a sampling interval of no greater than 196 μ m. The distance between tissue sections from mock-infected animals was no greater than 420 μ m.

Calculation of the rate of PrP^{Sc} spread. The rate of PrP^{Sc} spread following sciatic nerve inoculation was calculated by dividing the distance between the inoculation site and specific CNS structures by the number of days postinoculation (p.i.) when PrP^{Sc} was first detected. The distance of a given structure from the point of inoculation on the sciatic nerve to the middle of the structure was measured in millimeters. The HY TME agent-infected animals were sacrificed every week beginning at 1 week p.i. until the onset of clinical signs, and the DY TME agent-infected animals every week beginning at 12 weeks p.i. until the onset of clinical signs. Student's *t* test was performed to compare the average rates of PrP^{Sc} spread using the Prism 4.0 (for Macintosh) software program (GraphPad Software, Inc., San Diego, CA).

RESULTS

Initial DY and HY PrP^{Sc} spread from the sciatic nerve. To determine the initial deposition of DY and HY PrP^{Sc} following sciatic nerve inoculation, the DRG, sympathetic ganglia, and lumbar spinal cord were analyzed by PrP^{Sc} immunohistochemistry. Following inoculation of the DY TME agent, DY PrP^{Sc} was detected at 63 days p.i. in the neurons of the DRG, ipsilateral to the side of inoculation (Fig. 1B). Up to 63 days p.i., PrP^{Sc} was not detected in neurons of the sympathetic chain (Fig. 1E) or lamina IX of the lumbar spinal cord (Fig. 1H). DY PrP^{Sc} was first detected in lamina IX of the lumbar spinal cord associated with ventral motor neurons (VMNs) at 70 days post-DY TME agent inoculation of the sciatic nerve (data not shown).

In animals inoculated with the HY TME agent, PrP^{Sc} was first detected in the sensory neurons of the DRG 7 days p.i. ipsilateral to the side of inoculation (Fig. 1C). At 7 days p.i.,

TABLE 3. Temporal and spatial distribution of PrP^{Sc} in brain and brainstem following sciatic nerve inoculation with HY TME agent

CNS region	PrP ^{Sc} immunostaining at indicated no. of days postinfection ^a					
	21	28	35	42	49	56
Medulla: pons						
Reticular formation	0	0	+	++	+++	+++
Lateral vestibular nucleus ^b	0	+ ^c	++	+++	++++	++++
Cerebellum						
Interposed nucleus ^b	0	0	+ ^c	++	+++	+++
Mesencephalon						
Red nucleus ^b	0	+ ^d	+ ^d	++	+++	+++
Diencephalon						
Reticular thalamic nucleus ^b	0	0	0	+ ^{d,e}	+ ^d	+ ^d
Ventroposterior thalamic nucleus ^b	0	0	0	0	+ ^d	+ ^d
Telencephalon						
Hind limb cortex ^b	0	0	0	0	+ ^d	+++

^a Relative intensities of PrP^{Sc} immunostaining: 0, none; +, rare; ++, weak; +++, moderate; +++++, heavy.

^b Structure was previously reported to be PrP^{Sc} positive (2).

^c Asymmetrical staining pattern ipsilateral to inoculation site.

^d Asymmetrical staining pattern contralateral to inoculation site.

^e PrP^{Sc} was detected in one of three animals examined.

PrP^{Sc} was not detected in the neurons of the sympathetic chain (Fig. 1F) or VMNs of the lumbar spinal cord (Fig. 1I). As previously reported, the initial deposition in VMNs of the lumbar spinal cord was at 14 days after HY TME agent inoculation of the sciatic nerve (data not shown) (3). These data indicate that PrP^{Sc} detection in the DRG precedes PrP^{Sc} deposition in both the sympathetic ganglia and lumbar spinal cord following sciatic nerve inoculation with either the HY or DY TME agent.

DY and HY PrP^{Sc} spread in the brain and brainstem following sciatic nerve inoculation. To investigate and compare the spread of the two TME strains in the CNS, hamsters were inoculated in the sciatic nerve with either the DY or HY TME agent and tissues were examined at weekly intervals. PrP^{Sc} accumulated in progressively more rostral CNS structures as the length of time p.i. increased (Tables 2 and 3).

In hamsters inoculated in the sciatic nerve with the DY TME agent, PrP^{Sc} was first detected bilaterally in the reticular formation of the medulla 84 days after inoculation and increased in abundance by 139 days p.i. (Fig. 2O; Table 2). Beginning at 111 days p.i., DY PrP^{Sc} was detected ipsilaterally in the lateral vestibular nucleus and the interposed nuclei of the cerebellum, and it increased in abundance by 139 days p.i. (Fig. 2K; Table 2). At 127 days p.i., DY PrP^{Sc} was detected bilaterally in the ventrolateral part of the red nucleus and in layers IV to VI of the hind limb motor cortex (Table 2). In one of three animals killed at 127 days p.i., there was weak staining for DY PrP^{Sc} in the contralateral reticular and ventroposterior nuclei of the thalamus (Table 2). The staining was notably more abundant in the contralateral red nucleus, the contralateral thalamus, and the contralateral cortex at both 127 and 139 days p.i. (Fig. 2C and G and data not shown). Each of the labeled structures contains neurons that give rise to axons

FIG. 2. PrP^{Sc} deposition in hind limb motor cortex, red nucleus, lateral vestibular nucleus, and reticular formation following DY or HY TME agent inoculation of the sciatic nerve. Nissl staining (A) of the contralateral hind limb motor cortex at low power reveals the cortical layers of the hind limb and lateral agranular cortex. PrP^{Sc} immunostaining in cortical layers four (IV), five (V), and six (VI) of the contralateral hind limb motor cortex located in a similar area to the boxed region in panel A at 139 days post-DY TME inoculation (C) or 56 days post-HY TME inoculation (D) is shown. Nissl staining (E) of mesencephalon with both red nuclei outlined with dashed lines is shown. PrP^{Sc} immunostaining of the mesencephalon at 139 days post-DY TME inoculation (G) or 42 days post-HY TME inoculation (H) is shown. Note that at these time points postinfection, the majority of PrP^{Sc} immunoreactivity is confined to the ventrolateral portion of the contralateral red nucleus. Low power image of a nissl-stained section (I) of the ipsilateral pons with the lateral vestibular nucleus (LVN) outlined with dashed lines. PrP^{Sc} immunostaining on neurons of the LVN located in an area similar to the boxed region in panel G at 139 days post-DY TME inoculation (K) or 42 days post-HY TME inoculation (L) is shown. A low power image of a nissl-stained (M) section of the medulla shows the reticular formation outlined with dashed lines. PrP^{Sc} immunostaining on the gigantocellular reticular neurons located in an area similar to the boxed region in panel M at 139 days post-DY TME inoculation (O) or at 56 days post-HY TME inoculation (P) is shown. In mock-infected hamsters, PrP^{Sc} was not detected in the hind limb motor cortex (B), red nucleus (F), lateral vestibular nucleus (J), or reticular formation (N). Abbreviations: 4V, fourth ventricle; 7N, facial nucleus; Py, pyramidal tract. Scale bar, 100 μ m.

which form the descending motor tracts or in the case of the interposed nucleus of the cerebellum receives a collateral branch from a descending motor axon (16). The staining in the thalamus is likely due to transsynaptic spread from cortical neurons, similar to that which has been reported previously following HY TME agent sciatic nerve inoculation (2). Thus, DY PrP^{Sc} appears to be selectively transported retrogradely up the axons of the reticulospinal tract, the vestibulospinal tract, the rubrospinal tract, and the corticospinal tract following sciatic nerve inoculation (Fig. 3).

We performed PrP^{Sc} immunohistochemistry analysis on the brain and brainstem of hamsters inoculated in the sciatic nerve with the HY TME agent to confirm and extend previous findings (2). The temporal and spatial spread of PrP^{Sc} to the lateral vestibular nucleus, interposed nucleus, red nucleus, thalamic

nuclei, and hind limb motor cortex is consistent with the retrograde transport of the HY TME agent along the vestibulospinal tract, the rubrospinal tract, and the corticospinal tract, as reported previously (Table 3; Fig. 2D, H, and L) (2). In addition, HY PrP^{Sc} was identified bilaterally in the reticular formation of the medulla beginning at 35 days p.i. and increased in intensity at later time points p.i. (Table 3; Fig. 2P), which has not been reported previously (2). HY PrP^{Sc} appears to be retrogradely transported up the reticulospinal tract, identical to what was seen for DY PrP^{Sc} following sciatic nerve inoculation (Fig. 2).

HY and DY TME PrP^{Sc} accumulated in the same structures within the spinal cord, brainstem, and brain, consistent with retrograde axonal transport via the same four descending motor tracts (Tables 2 and 3; Fig. 2 and 3).

FIG. 3. The DY and HY TME agents are selectively transported along four descending motor tracts following sciatic nerve inoculation. Infection of VMNs of the lumbar spinal cord results in retrograde transport of both the DY and HY TME agents via the corticospinal, rubrospinal, vestibulospinal, and reticulospinal descending motor tracts. Abbreviations: RF, reticular formation; LVN, lateral vestibular nucleus; RN, red nucleus; MC, hind limb motor cortex; DRG, dorsal root ganglia.

Rates of DY and HY PrP^{Sc} spread. In hamsters inoculated with the DY TME agent, the rates of spread from the inoculation site in the sciatic nerve to ventral motor neurons in the lumbar spinal cord, the lateral vestibular nucleus, the red nucleus, and the hind limb motor cortex were 0.92, 1.43, 1.00, and 1.03 mm/day, respectively, with an average rate of spread of 1.10 ± 0.11 mm/day (Table 4). This rate of spread is significantly

TABLE 4. Strain-specific rate of prion spread from the site of inoculation to CNS structures					
Strain	Rate of spread (mm/day) from sciatic nerve inoculation site to indicated structure				Mean
	Ventral motor neurons	Lateral vestibular nucleus	Red nucleus	Hind limb motor cortex	
DY TME	0.92	1.43	1.00	1.03	1.10 ± 0.11 ^a
HY TME	4.61	4.29	4.52	3.12	4.14 ± 0.35 ^b

^a Average ± standard error of the mean.
^b Significantly different (*P* < 0.01) from the average rate of DY PrP^{Sc} spread.

TABLE 5. Presence of PrP^{Sc} in the brain and brain stem upon development of clinical disease following sciatic nerve inoculation with the HY or DY TME agent

CNS region	Presence of PrP ^{Sc} after inoculation of ^a :	
	HY TME	DY TME
Brain stem		
Trigeminal motor nucleus	+	+
Trigeminal principal sensory nucleus	+	+
Facial motor nucleus	+	+
Hypoglossal nucleus	+	+
Forebrain		
Hippocampus		
Dentate gyrus	0	+
Hippocampus proper	0	+
Subiculum	0	+
Thalamus	+	+
Hypothalamus	+	+
White matter		
Cerebellar white matter	+	+
Corpus callosum	0	+
Anterior commissure	0	+
Cingulum	0	+
External capsule	0	+

^a +, present; 0, absent.

lower (*P* < 0.01) than the rate of PrP^{Sc} spread to the same structures in HY TME agent-infected hamsters (Table 4).

DY and HY PrP^{Sc} distribution in the brain and brainstem at terminal disease following sciatic nerve or intracerebral inoculation. The patterns of PrP^{Sc} distribution in HY and DY TME agent-infected animals following sciatic nerve inoculation are different at the onset of clinical signs (67 ± 3 and 235 ± 2 days, respectively) (Table 5). PrP^{Sc} was detected in every structure examined at the onset of clinical signs in the DY TME agent-infected hamsters (Table 5). The pattern of PrP^{Sc} deposition at the onset of clinical signs in the hamsters inoculated with the HY TME agent was more restricted compared to that observed in hamsters inoculated with the DY TME agent (Table 5). Specifically, PrP^{Sc} was not detected in the hippocampus or select white matter structures of hamsters inoculated with the HY TME agent (Fig. 4B and E; Table 5).

PrP^{Sc} immunoreactivity was detected as coarse punctate deposits throughout the hippocampus and white matter upon development of clinical disease following i.c. inoculation with the HY TME agent (Fig. 4C and F), indicating that the lack of HY PrP^{Sc} immunoreactivity following sciatic nerve inoculation is not due to an inherent inability of the HY TME agent to replicate in these structures.

DISCUSSION

This study is the first to compare the route of spread of two prion strains over time. We inoculated two prion strains into the same peripheral target, the sciatic nerve, to test the hypothesis that the characteristic neuropathology of prion strains is due to strain-specific transport along different neuroanatomical tracts. Interestingly, both TME strains were transported to the same structures through the majority of the incubation periods. The subtle temporal differences of initial

FIG. 4. Regional deposition of HY PrP^{Sc} is influenced by the route of infection. In mock-infected hamsters, PrP^{Sc} was not detected in the hippocampus (A) or corpus callosum (D). PrP^{Sc} is not detected in the hippocampus (B) or corpus callosum (E) of hamsters inoculated in the sciatic nerve (i.s.c.) with the HY TME agent at clinical disease, in contrast to results for hamsters inoculated with the HY TME agent i.c., where PrP^{Sc} is present as coarse punctate deposits in both structures (C and F). Abbreviations: CA3, field CA3 of hippocampus; PoDG, polymorphic layer of dentate gyrus; CX, cerebral cortex; CC, corpus callosum; SO, stratum oriens. Scale bar, 100 μ m.

PrP^{Sc} detection in certain CNS structures between the TME strains does not alter the conclusion that HY and DY TME are both transported along the four same descending motor tracts. At later time points p.i., widespread PrP^{Sc} deposition resulted in an inability to unequivocally determine which neuroanatomical pathway was used. As a consequence, the possibility cannot be excluded that the two TME strains use different pathways at late time points p.i.

Retrograde centripetal prion transport appeared to be the primary mode of prion spread through the majority of the incubation period. The initial spread of PrP^{Sc} for both TME strains was retrograde to sensory neurons in the DRG and motor neurons in the ventral horn of the spinal cord (Fig. 1 and 3). Through the majority of the incubation period of both the HY and DY TME infections, PrP^{Sc} was detected only in nuclei of the origin of the descending motor tracts (Tables 2 and 3; Fig. 2 and 3). During this same time period, there was no evidence of PrP^{Sc} deposition in structures associated with anterograde transport along ascending sensory pathways. This observation suggests that PrP^{Sc} located in dorsal root ganglia neurons has a decreased capacity to be anterogradely (versus retrogradely) transported (Fig. 2 and data not shown). The observed initial exclusive retrograde transport of HY and DY PrP^{Sc} extends and confirms observations in other studies that examined the early spread of prions (1, 2, 24, 32). It is not known if the preference for retrograde spread of PrP^{Sc} is due to specific directional transport along axons or to selective directional transport across synapses. However, the observed specificity of prion transport via descending motor tracts and strain-specific rates of spread suggests that initial centripetal prion transport is not passive diffusion along axons or oligodendrocytes (25). Detection of PrP^{Sc} in structures that are consistent with anterograde transport has been observed only

just prior to or during the clinical phase of disease (2, 30, 31). The reason for this observation is not known; however, it is possible that (i) the neurons responsible for centripetal spread are not infected until later time points p.i., (ii) anterograde prion transport is the result of passive diffusion along axons, or (iii) anterograde transport occurs only after agent replication reaches a certain level.

The results from this model system do not support a hypothesis that states differential susceptibility of neuronal populations to prion replication determines prion strain targeting. PrP^{Sc} was detected by immunohistochemistry in every structure examined upon development of clinical disease following sciatic nerve inoculation of the DY TME agent. It is therefore reasonable to propose that refractory populations of neurons do not exist (Tables 1 and 5). While the vast majority of CNS structures are PrP^{Sc} positive in animals infected with either prion strain, there are differences observed at the onset of clinical signs. The most notable difference is a lack of PrP^{Sc} in the hippocampus and select white matter structures following sciatic nerve inoculation with the HY TME agent (Table 5). The lack of HY PrP^{Sc} in these structures cannot be due to an inability of the HY TME agent to replicate in these neurons, since these structures are PrP^{Sc} positive following i.c. inoculation (Fig. 4) (4). Cumulatively, the results indicate that there are no neuronal populations that are specifically refractory to certain strains of prions.

The observed strain-specific differences in PrP^{Sc} deposition upon development of clinical disease may be due to the length of time that PrP^{Sc} has to spread in the CNS before the host succumbs. We propose it is likely that the more widespread distribution of PrP^{Sc} in hamsters inoculated with the DY TME agent than in those inoculated with the HY TME agent is because DY TME agent requires more time than the HY TME

agent to reach or to destroy the areas responsible for the onset of clinical disease (i.e., clinical target areas [CTAs]) (19, 21, 22). In this scenario, the host succumbs to disease (i.e., the CTAs are destroyed) prior to the spread of the HY TME agent to the hippocampus and white matter areas. Therefore, strain targeting may be better defined as the time at which the CTAs are infected and destroyed. It is this outcome that may influence the distribution and intensity of neuropathological changes upon development of clinical disease.

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