Transport of prion strains in the peripheral and central nervous system

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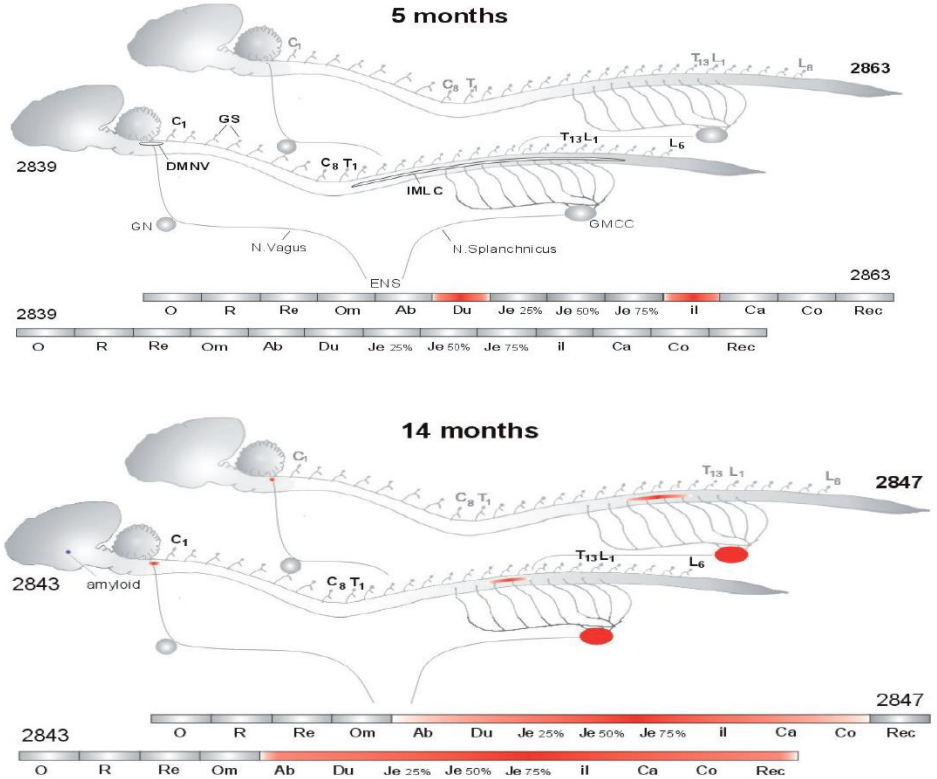
Prion pathogenesis

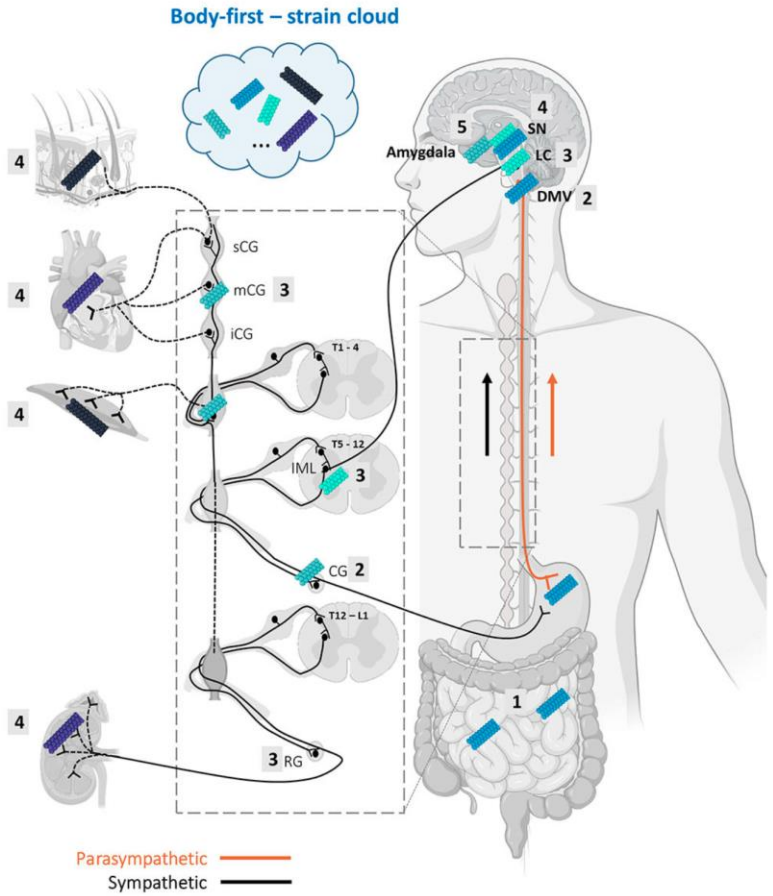
Scrapie

Oronasal → LRS → PNS → CNS



Van Keulen et al., 2000

Body-first synucleinopathy



Just et al., 2022

Prion pathogenesis

Sciatic nerve inoculation

- Can cause disease bypassing the requirement for extraneural prion replication.
- Rate of prion spread was 1-2mm per day, consistent with slow axonal transport.
- Prion titers in PNS were lower than CNS or LRS.

Journal of the Neurological Sciences, 1983, 61: 315–325
Elsevier

PATHOGENESIS OF MOUSE SCRAPIE

Evidence for Direct Neural Spread of Infection to the CNS after Injection of Sciatic Nerve

R.H. KIMBERLIN[1], SUSAN M. HALL[2] and CAROL A. WALKER[3]

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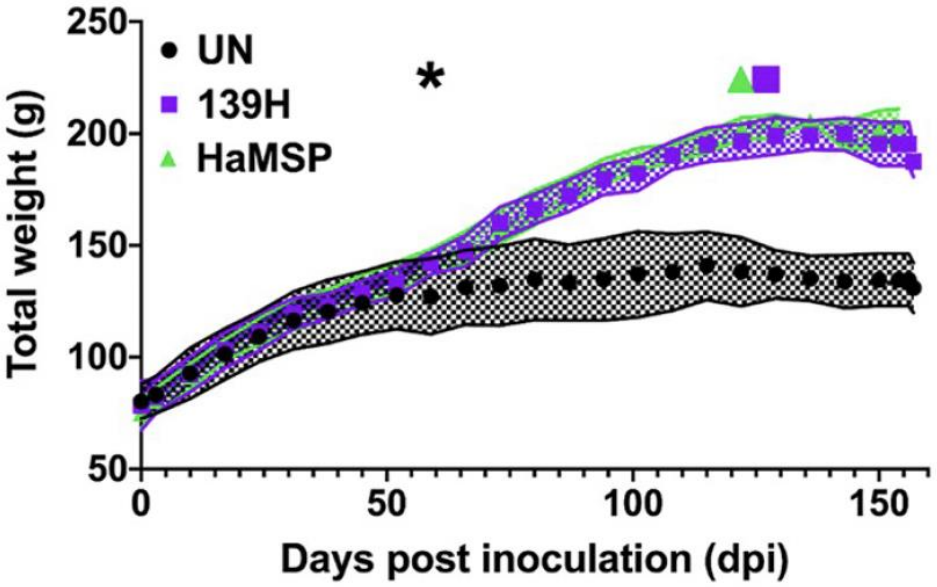
Alyssa Block


Benjamin Steadman

Prion pathogenesis

Sciatic nerve inoculation

Route, intra-	HY TME	139H	DY TME
Cerebral	58±3 (5/5)	123±3 (5/5)	179±3 (5/5)
Sciatic nerve	67±3 (5/5)	188±3 (5/5)	235±3 (5/5)
Peritoneal	101±3 (5/5)	225±3 (5/5)	>650 (0/5)



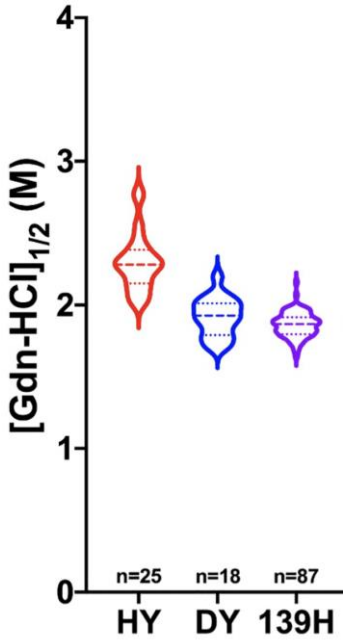
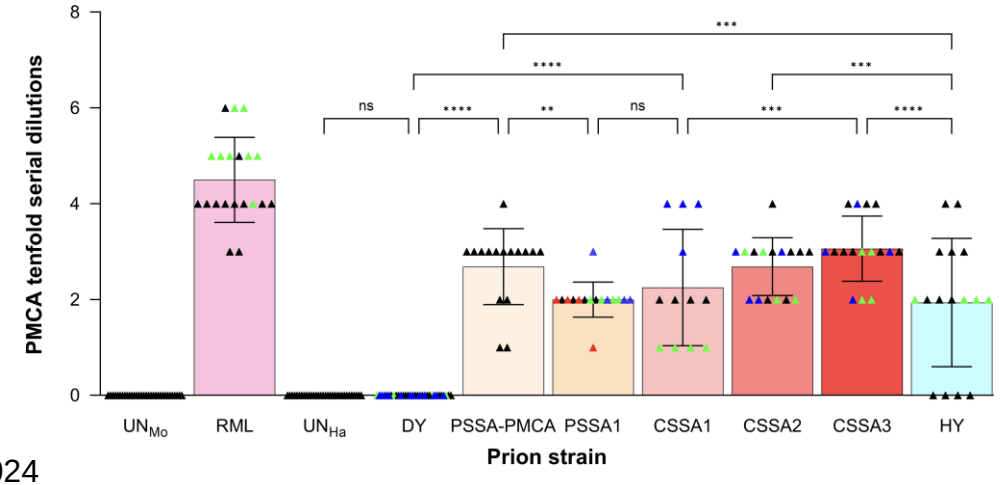


TABLE 3. Brain distribution of PrP^{TME} in HY and DY TME-infected hamsters

Brain region	HY TME		DY TME	
	Diffuse[a]	Plaque	Diffuse	Plaque
Hippocampus				
Dentate gyrus				
Granule layer	0	0	0	0
Polymorphic layer	++	++	+++	0
Molecular layer	0	0	++	0
Corpus callosum	0	+	0	+++
Occipital cortex[b]	++	++	+++	0
Medial geniculate nucleus	++++	0	0	0
Cerebellum				
Granule layer	++	+	+++	+
Purkinje layer	0	+	0	0
Molecular layer	0	++	++	+
White matter	0	++	0	++++
Deep nuclei	++++	++	0	+

[a] A scoring system was used to map the brain distribution of PrP^{TME}. The following symbols were used to describe the observed frequency and intensity of PrP^{TME} immunostaining: 0, none; +, rare; ++, mild; +++, moderate; ++++, heavy.

[b] HY PrP^{TME} staining was located primarily in the middle cortical layers, while DY PrP^{TME} staining was distributed in all cortical layers.

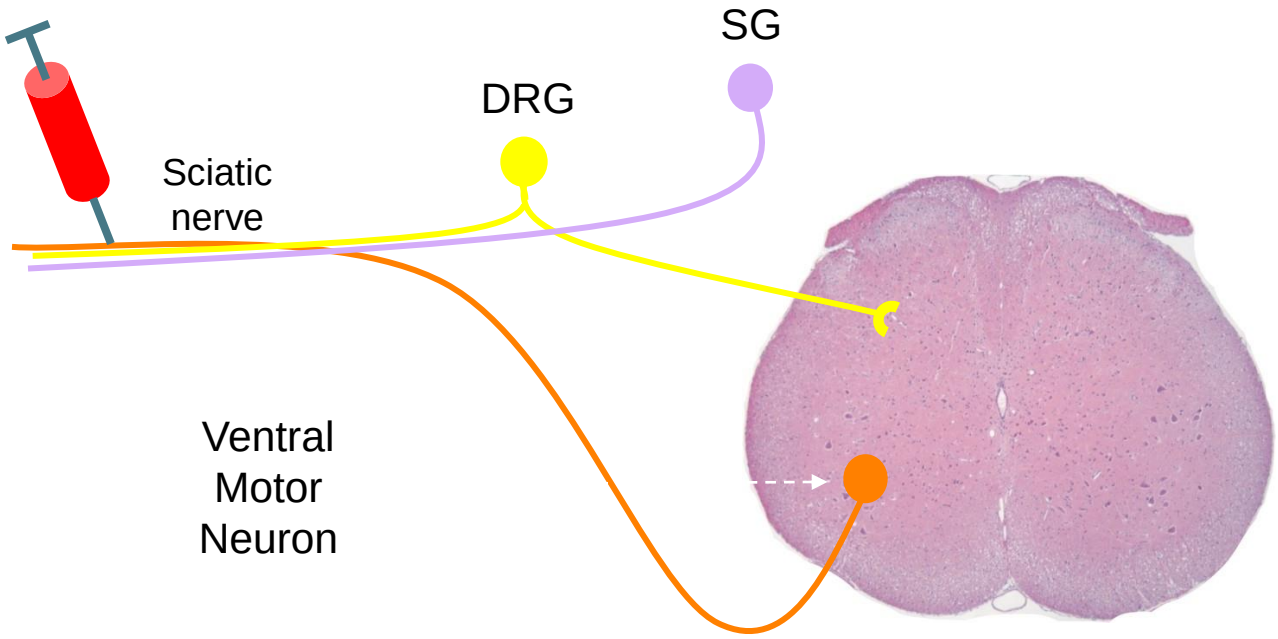


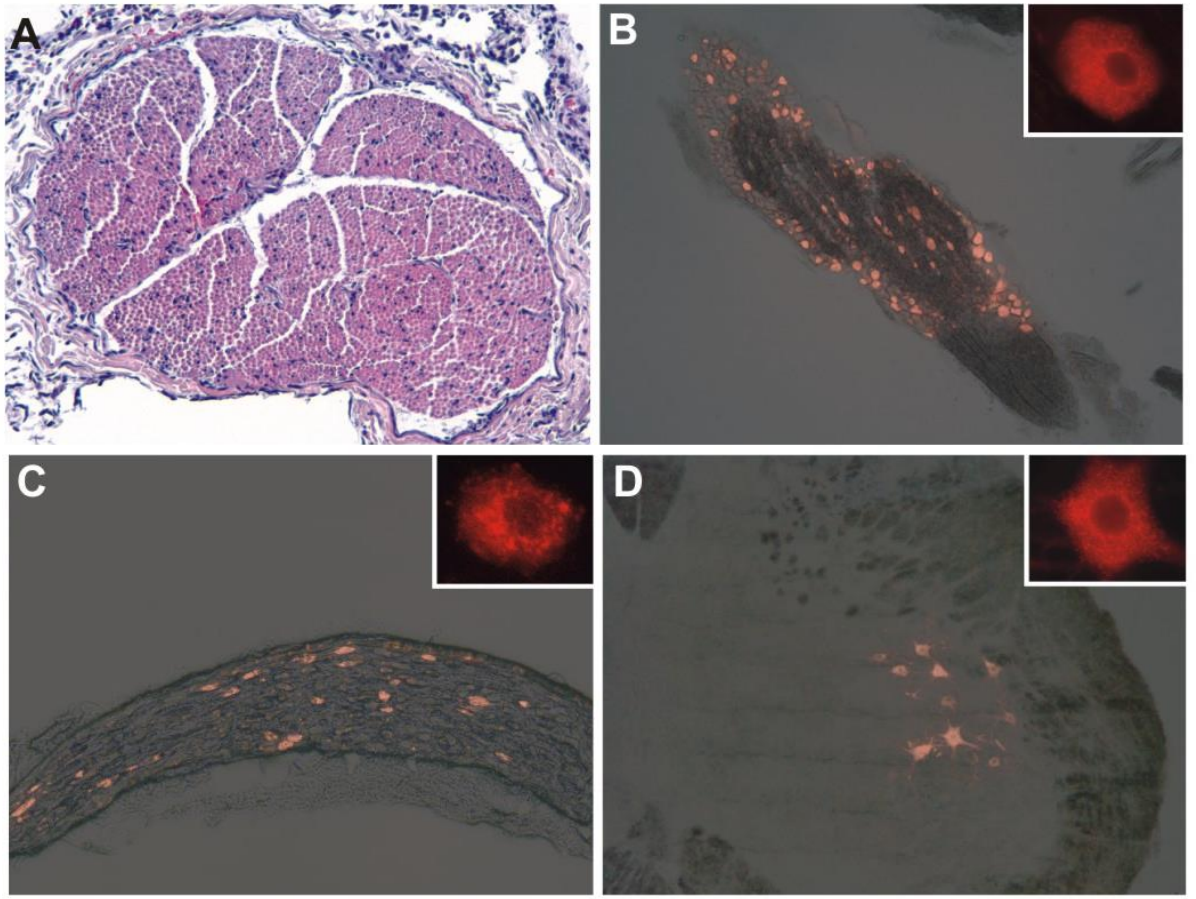
Bessen and Marsh, 1994; Ayers et al., 2011; Langenfeld et al., 2016; Block et al., 2021, Steadman et al., 2024

Jacob Ayers

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

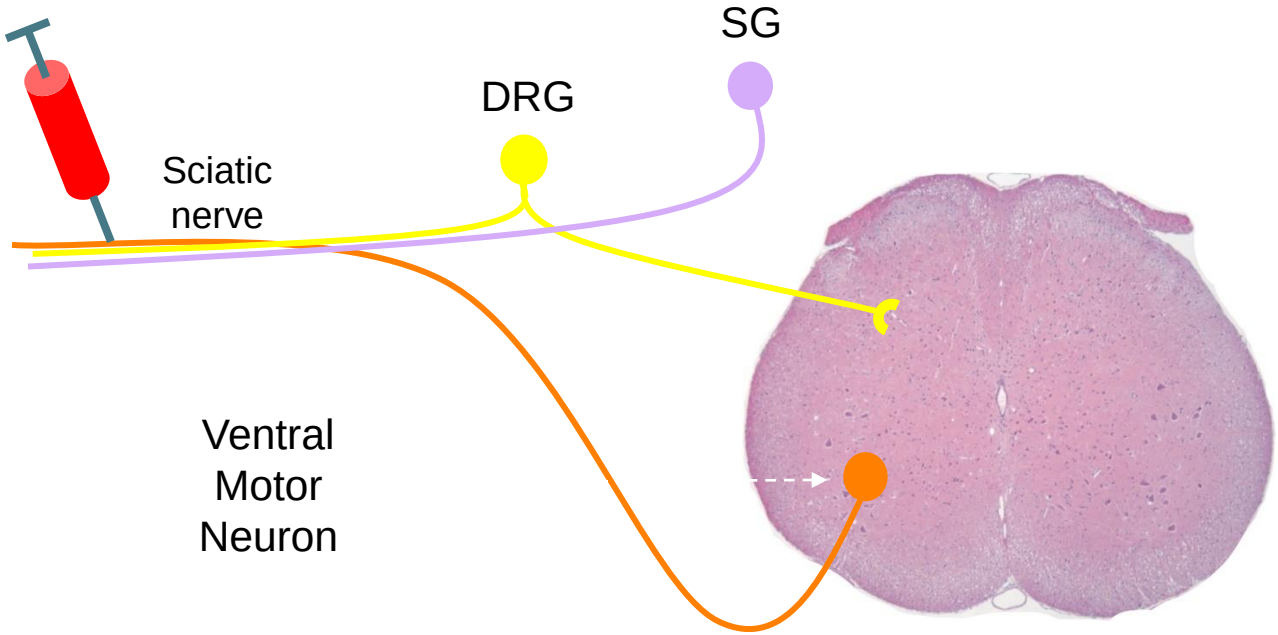


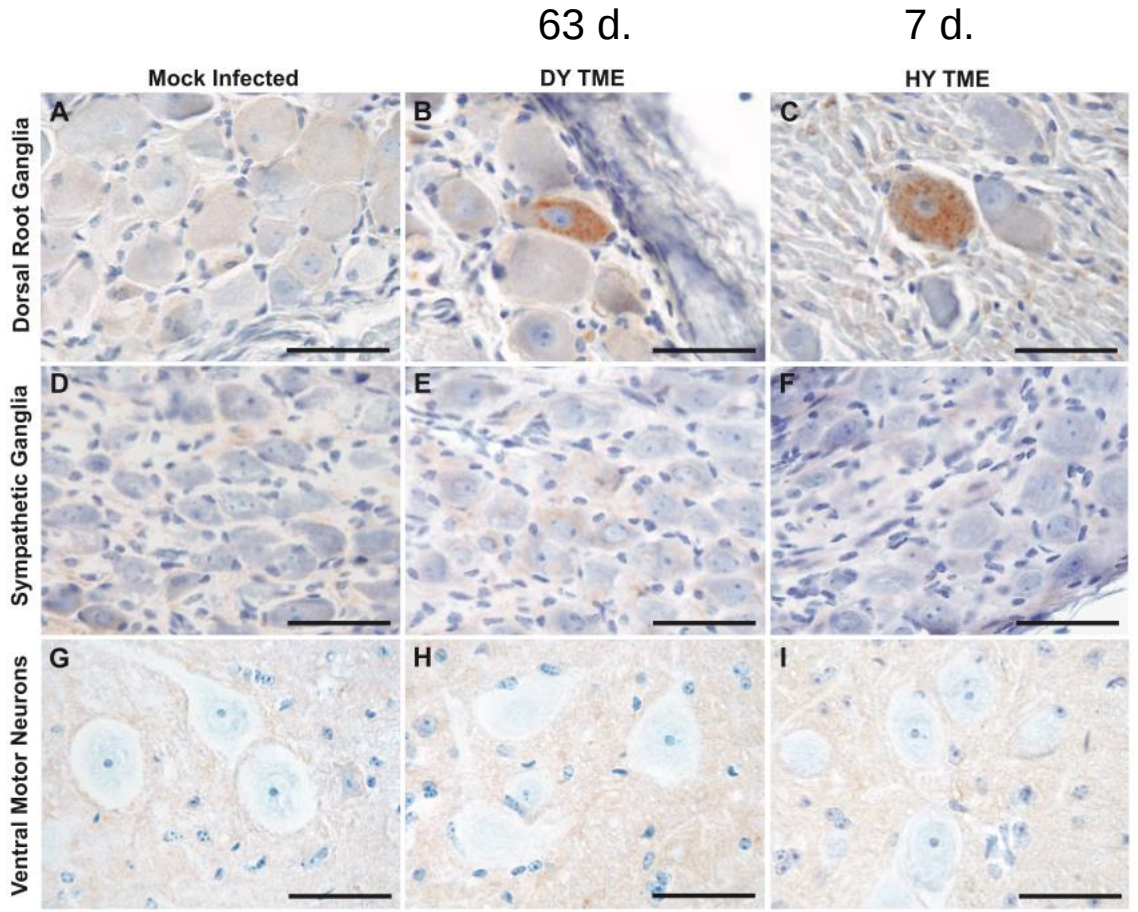


Ayers et al., 2009

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis



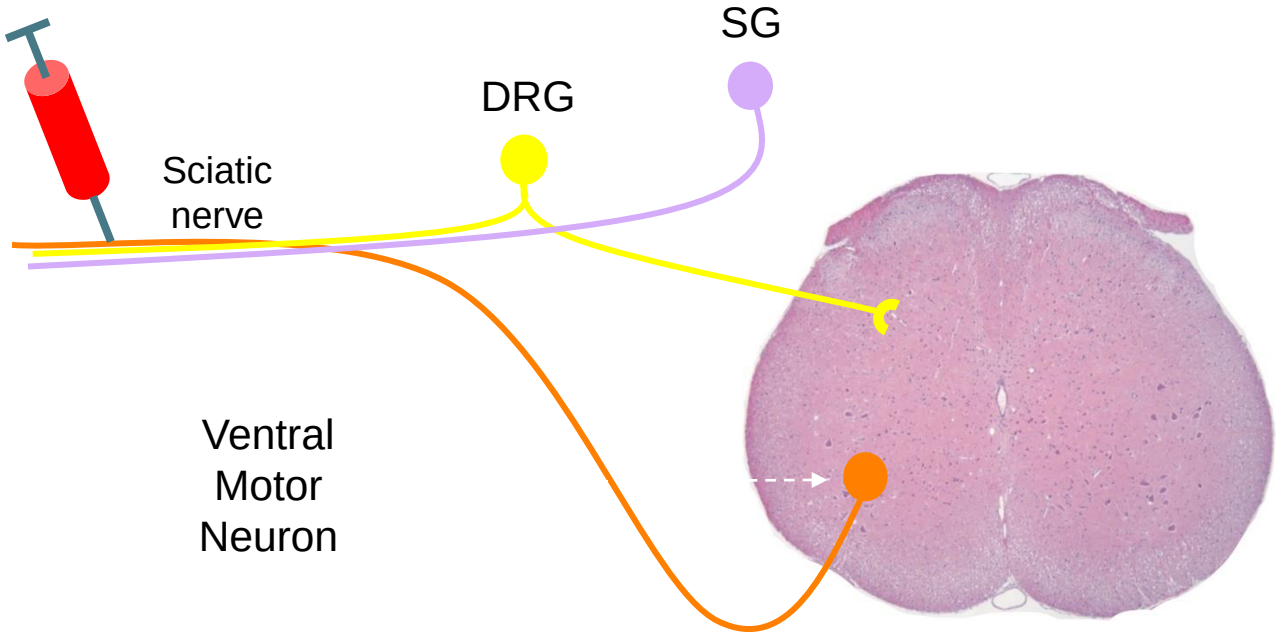


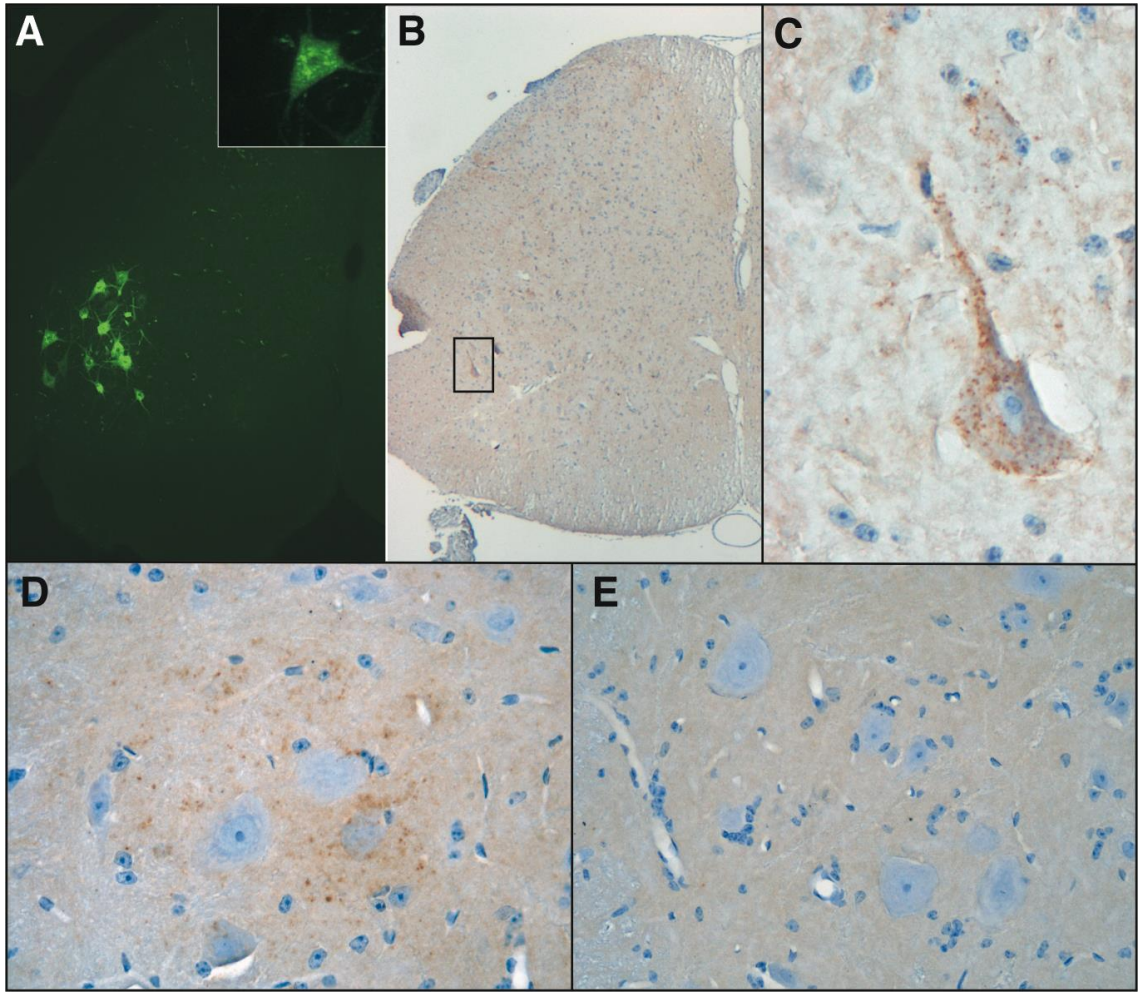
- PrP^{Sc} first detected in dorsal root ganglion

Ayers et al., 2009

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

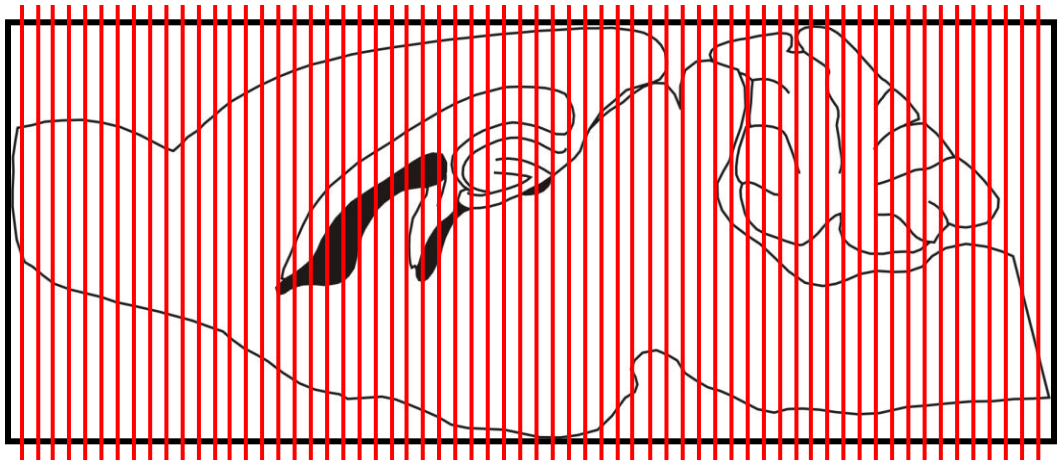


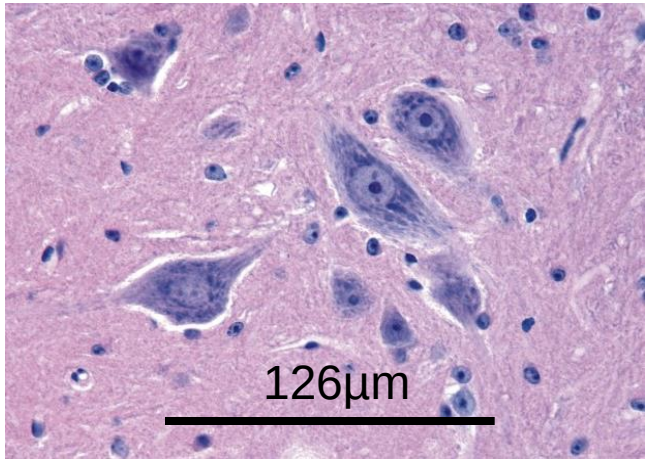


Ayers et al., 2009

Summary of PrP^{Sc} processed and analyzed tissue

- 3 infected and 2 mock infected per time point
- Size of brain ~ 9600µm
  - Cut at 7µm (total of ~1400 sections)



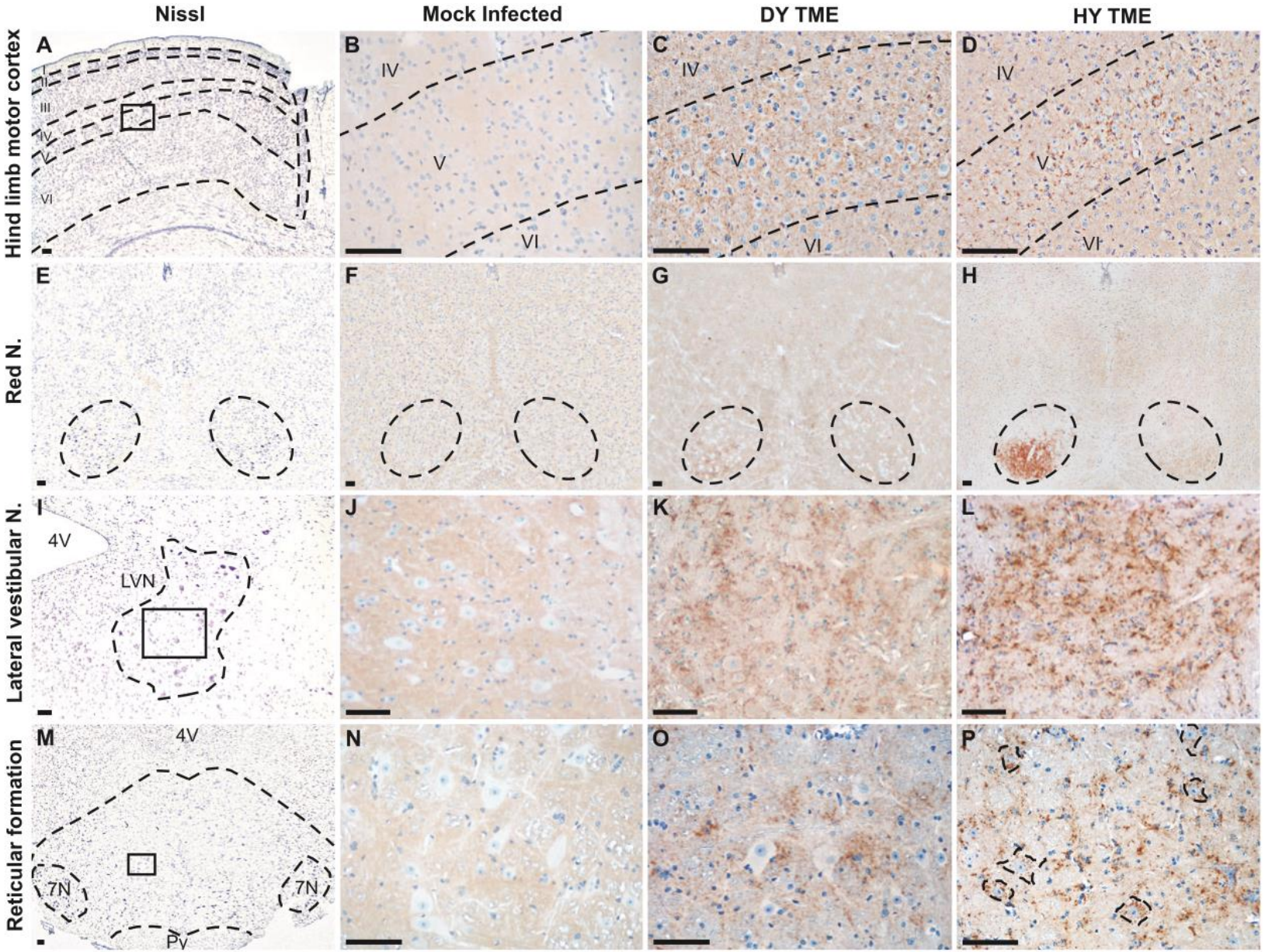


- PrP^{Sc} IHC on every 10^{th} slide.
  - Adjacent sections H&E and Nissl stained

- Maximum distance of 126µm between PrP^{Sc} IHC

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis



Ayers et al., 2009

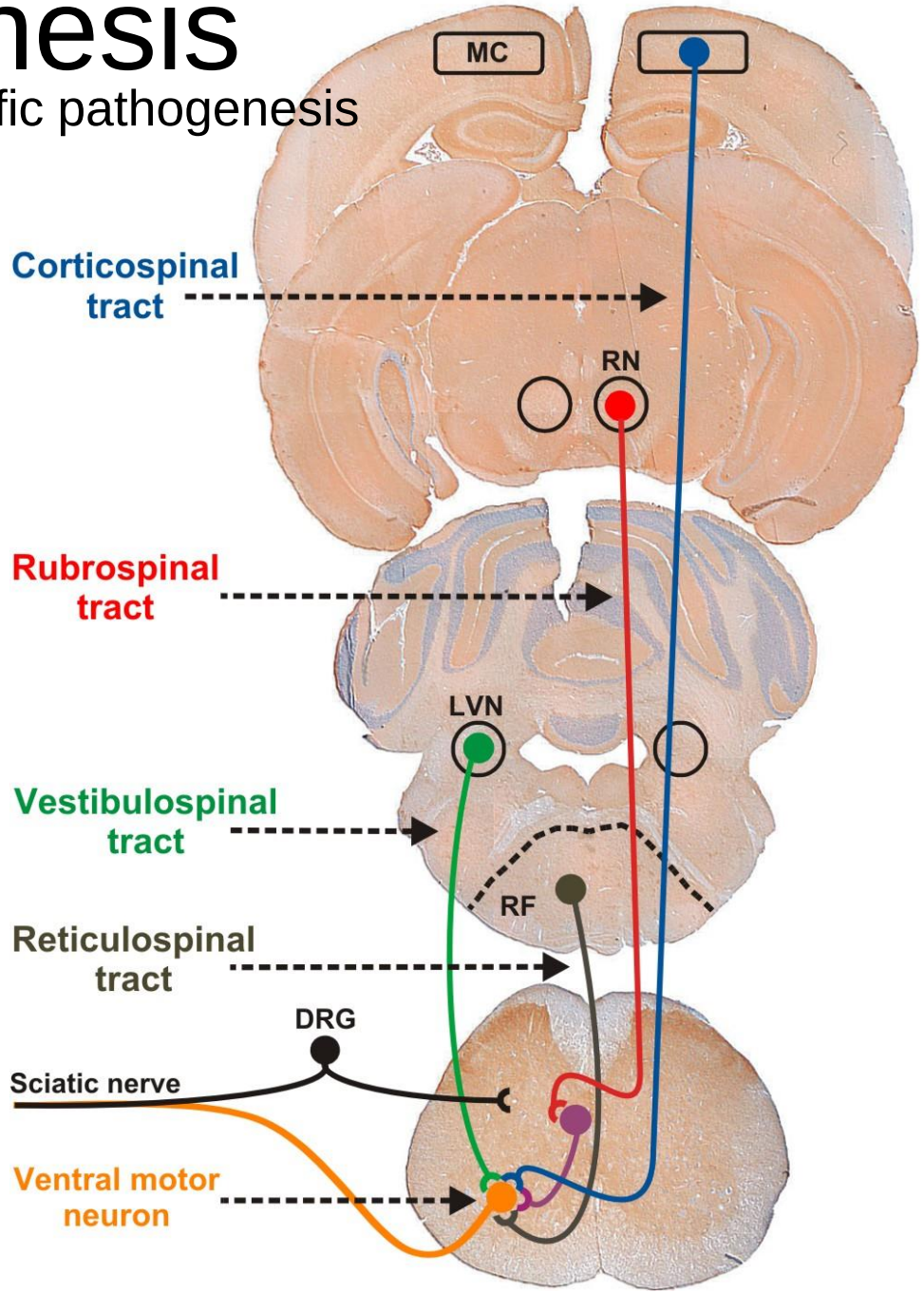
Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

HY TME

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.

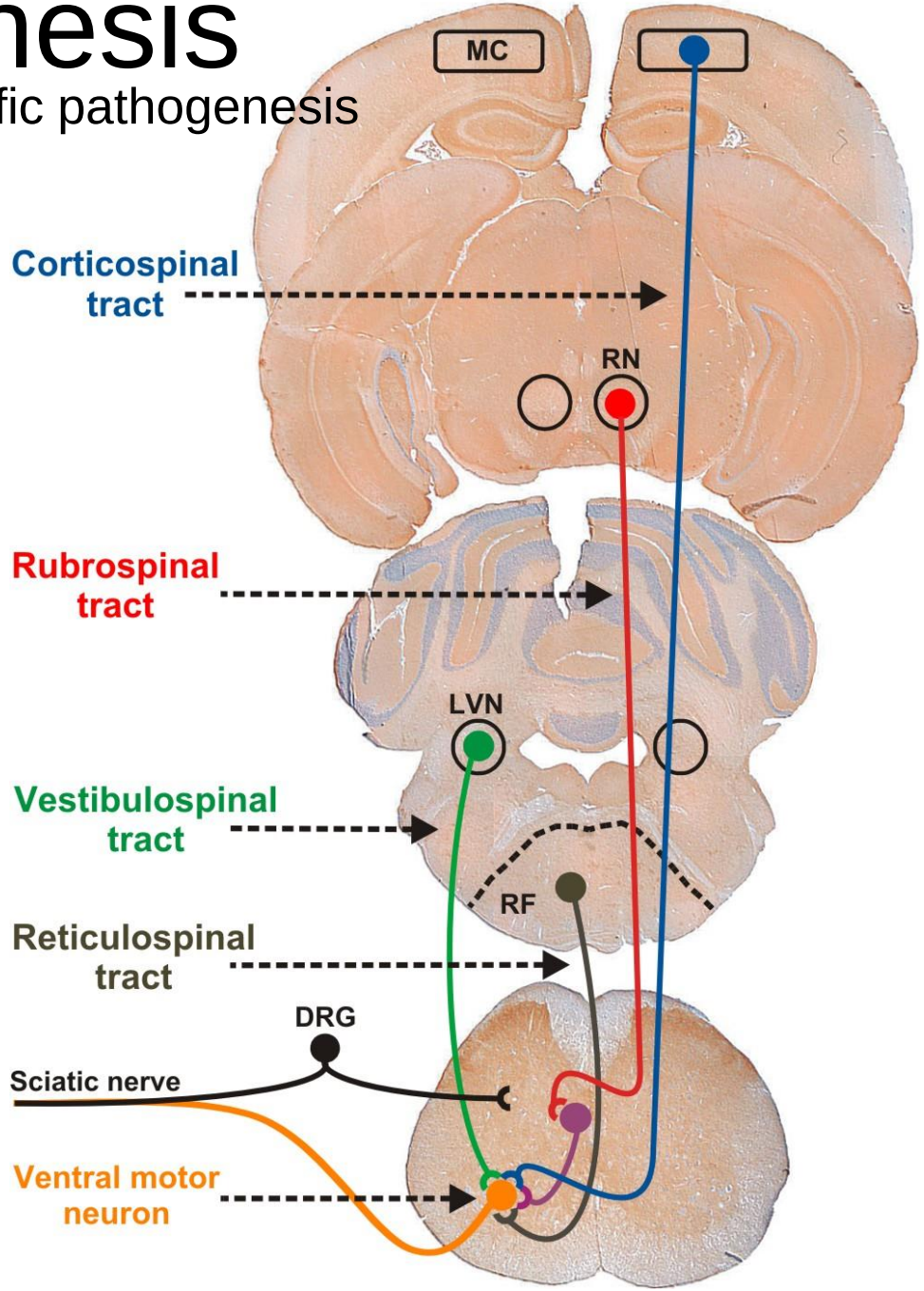


Bartz et al., 2002; 2007; Ayers et al., 2009; Langenfeld et al., 2016

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

- HY TME, DY TME and 139H prions infect VMNs ipsilateral to the side of inoculation and are transported via the *same* 4 descending motor tracts.
- The temporal and spatial spread of PrP^{Sc} differed between the strains as a function of days post infection.
- The temporal and spatial spread of PrP^{Sc} was similar when represented as a percentage of the incubation period.



Bartz et al., 2002; 2007; Ayers et al., 2009; Langenfeld et al., 2016

Prion pathogenesis

Sciatic nerve inoculation

Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways

Jacob I. Ayers[1] · Susan E. Fromholt[1] · Veronica M. O'Neal[1] · Jeffrey H. Diamond[1] · David R. Borchelt[1,2]

"In addition, a spatiotemporal study of these injections revealed a predictable spread of pathology to brain regions whose axons synapse directly on ventral motor neurons in the spinal cord, strongly supporting axonal transport as a mechanism of spread of the αS inducing, or seeding, factor."

Table 2 Spatiotemporal distribution and abundance of G85R-SOD1:YFP inclusion pathology following sciatic nerve inoculation with G85R-SOD1:YFP homogenate

	1 month	2 month-asym	2 month-sym	End-stage
Spinal cord				
Lumbar	–[a]	++	+++	+++
Thoracic	–	+	++	+++
Cervical	–	+	+	+++
Brain				
Ret. Form.	–	+	++	+++
Lat. Vest. Nuc.	–	+	+	++
Red. Nuc.	–	+	+	++
Periaq. gray	–	–	+	++
Sup. Coll.	–	–	+	++
Mot. Cx.	–	–	–	–

Localized Induction of Wild-Type and Mutant Alpha-Synuclein Aggregation Reveals Propagation along Neuroanatomical Tracts

Jacob I. Ayers,[a] Cara J. Riffe,[a] Zachary A. Sorrentino,[a] Jeffrey Diamond,[a] Eric Fagerli,[a] Mieu Brooks,[a] Ahmad Galaleldeen,[b,c] P. John Hart,[c,d] Benoit I. Giasson[a]

TABLE 1 Spatiotemporal distribution and abundance of αS pathology in M83$^{+/-}$ mice following sciatic nerve inoculation with mouse WT αS fibs

	Relative abundance of αS inclusion pathology[a]		
Body site	**1 mo p.i.**	**2 mo p.i.**	**Clinical (3.9 ± 0.1 mo p.i.)**
DRG			
Ipsilateral	+	++	++
Contralateral	–	–	+
Spinal cord			
Lumbar	+	++	+++
Thoracic	–	+	+++
Cervical	–	–	+++
Brain	–		
Reticular formation	–	++	+++
Lateral vestibular nucleus	–	–	++
Red nucleus	–	+	+++
PAG	–	+	+++
Motor cortex	–	–	+

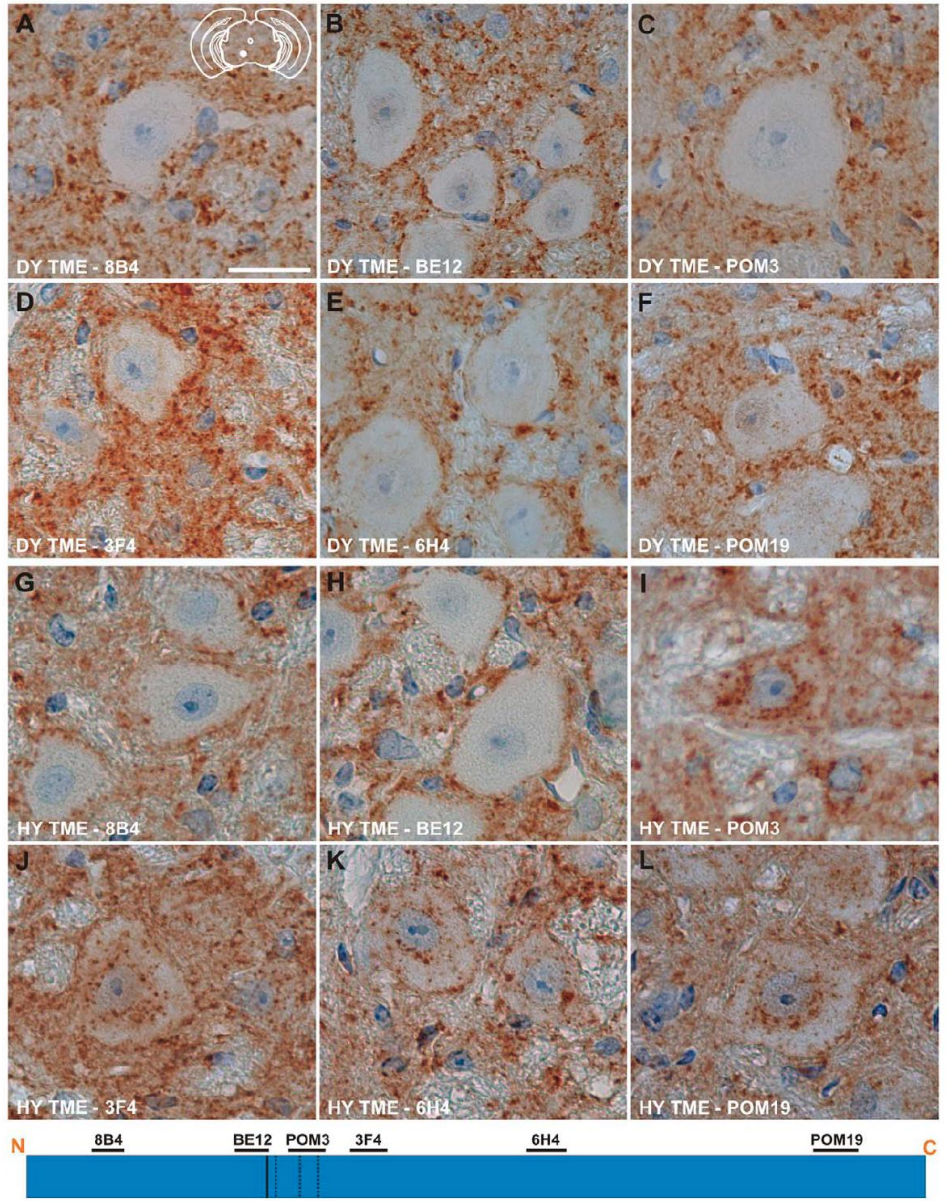
[a] –, none; +, rare; ++, numerous; +++, abundant and widespread.

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

- Strain-specific differences in PrP^{Sc} deposition patterns in neurons
- DY characterized by PrP^{Sc} deposition in dendritic arborization.

Strain	Incubation period (days)	PrP^{Sc} amplifcation rate (Amp. Co.)	PrP^{Sc} conformational stability (Gdn-HCl / SDS)	PrP^{Sc} truncation profile
263K HaCWD HY TME	Short (<65)	Fast (2-20)	High (≥1.15/≥0.78)	8B4 BE12 POM3 3F4 6H4 8H4 POM19 N C Neuropil Neuronal somata Astrocytes Microglia
22AH 22CH 139H ME7H	Long (>135)	Slow (0.02)	Low (≤1.02/≤0.53)	8B4 BE12 POM3 3F4 6H4 8H4 POM19 N C Neuropil N.S. Astrocytes Microglia
DY TME	Long (170)	Slow (0.02)	Low (0.43/0.53)	8B4 BE12 POM3 3F4 6H4 8H4 POM19 N C Neuropil N.S. Astrocytes Microglia



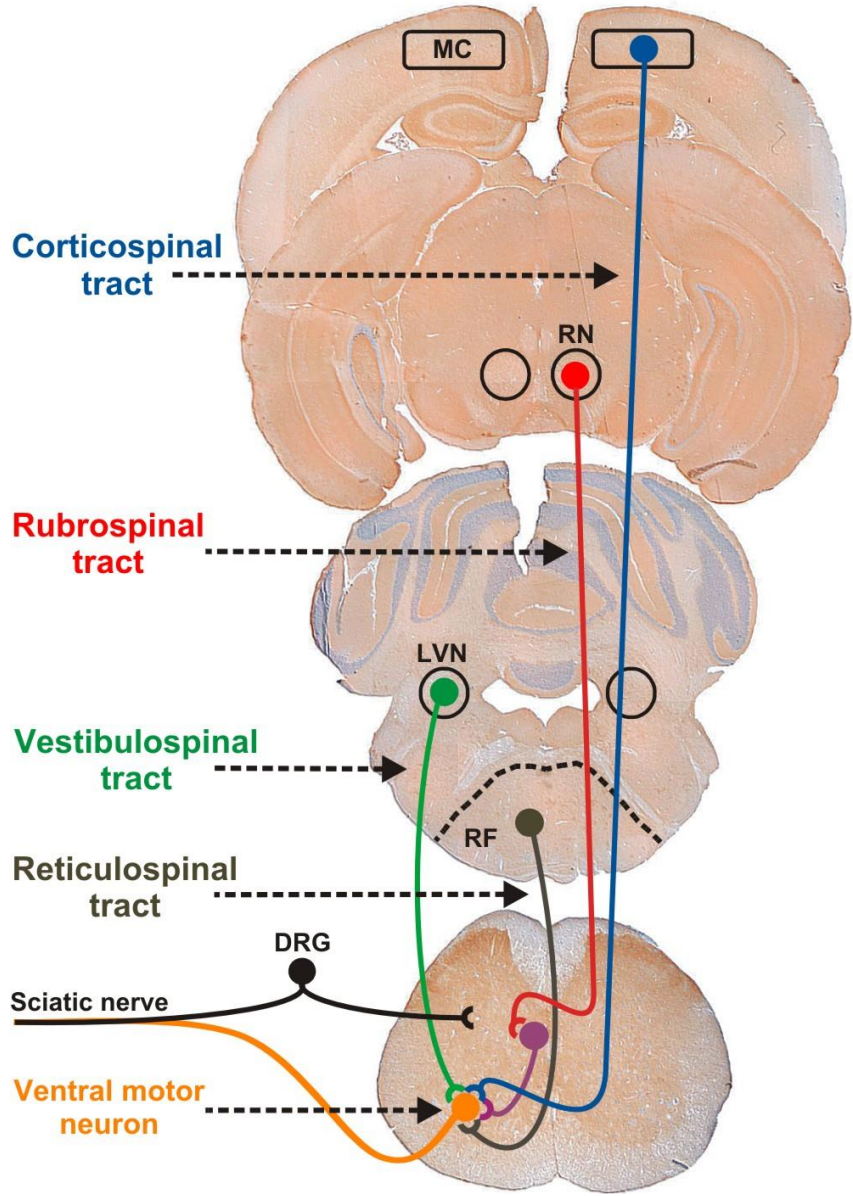
Ayers et al., 2011

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

	Ventral motor neuron	Lateral vestibular nucleus	Red nucleus	Hind limb motor cortex	Overall
HY TME	4.61	4.29	4.52	3.12	4.14±0.35
139H	1.29	1.60	2.59	1.75	1.80±0.27
DY TME	0.92	1.43	1.00	1.03	1.10±0.11

- Rate of spread is consistent with slow axonal transport
- Strain-specific rates of spread observed
- Correspond with tempo of disease

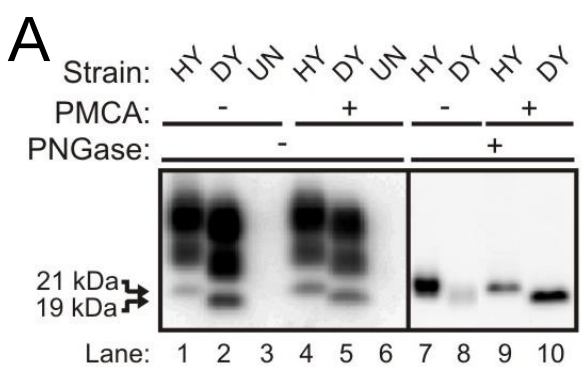


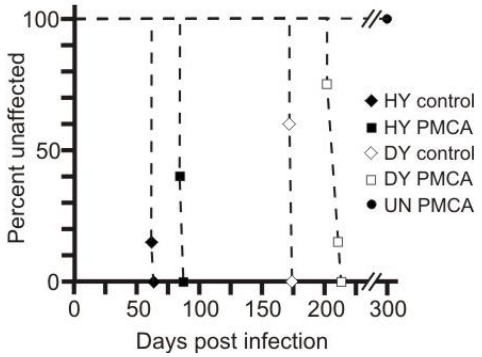
Ayers et al., 2009; Langenfeld et al., 2016

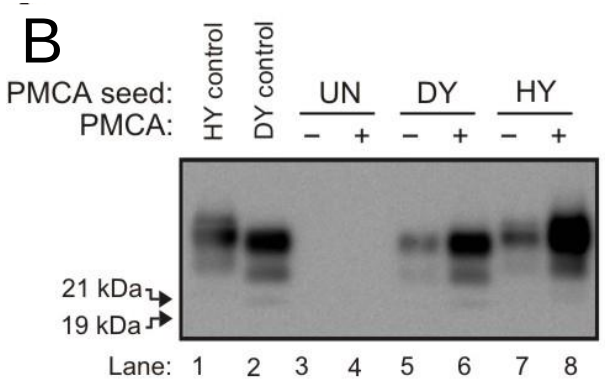
Ronald Shikiya

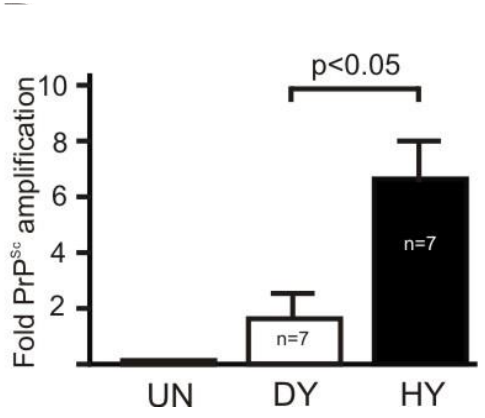
Protein Misfolding Cyclic Amplification

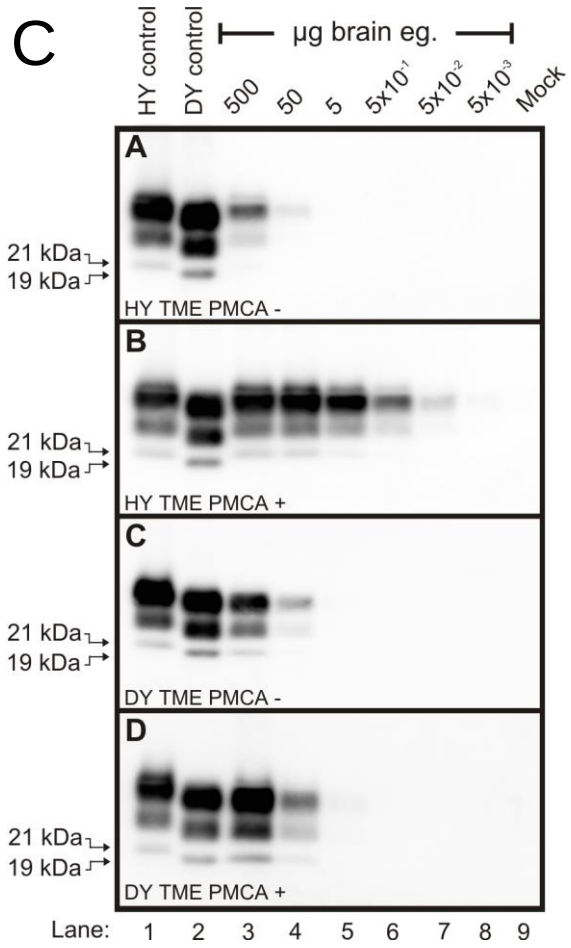
Recapitulates strain properties











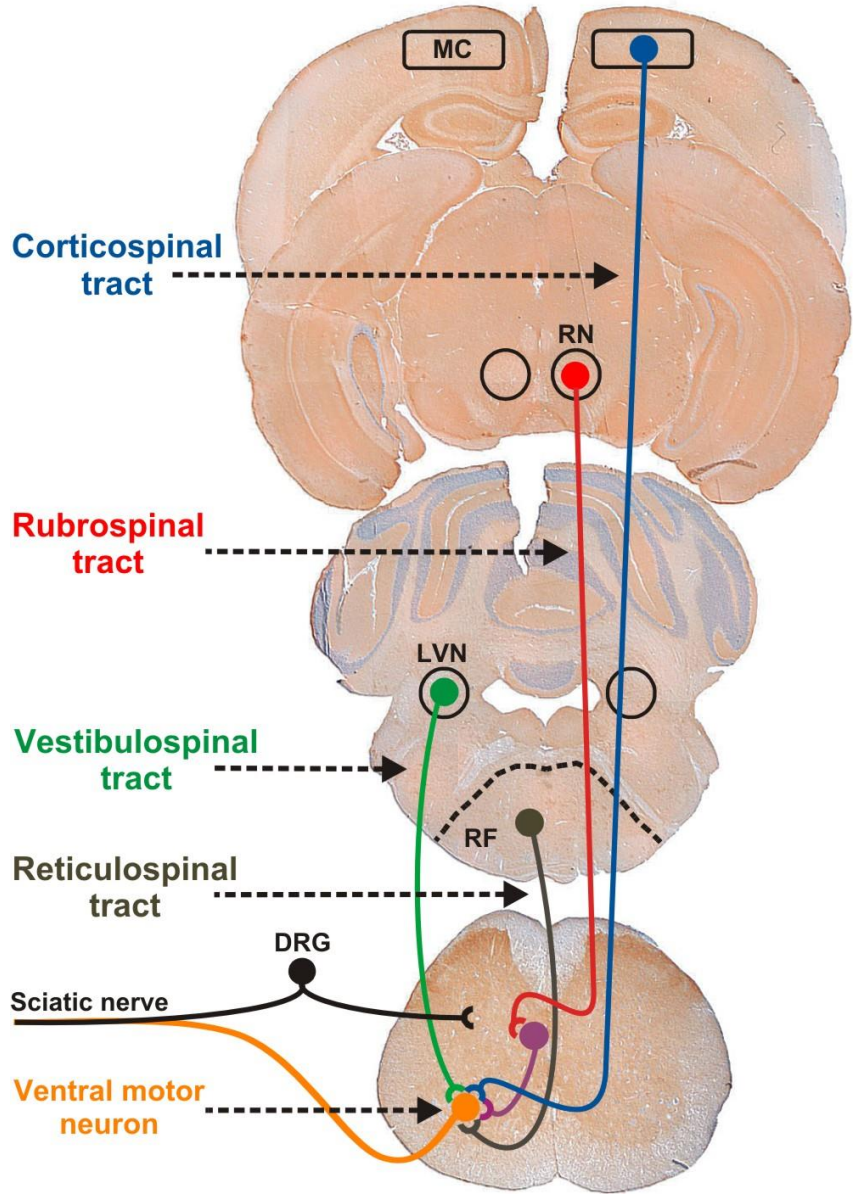
Shikiya et al., 2010; 2011; Ayers et al., 2011

Prion pathogenesis

Sciatic nerve inoculation – Strain specific pathogenesis

	Ventral motor neuron	Lateral vestibular nucleus	Red nucleus	Hind limb motor cortex	Overall
HY TME	4.61	4.29	4.52	3.12	4.14±0.35
139H	1.29	1.60	2.59	1.75	1.80±0.27
DY TME	0.92	1.43	1.00	1.03	1.10±0.11

- Rate of spread is consistent with slow axonal transport
- Strain-specific rates of spread observed
- Correspond with tempo of disease
- Rate of transport vs. rate of spread



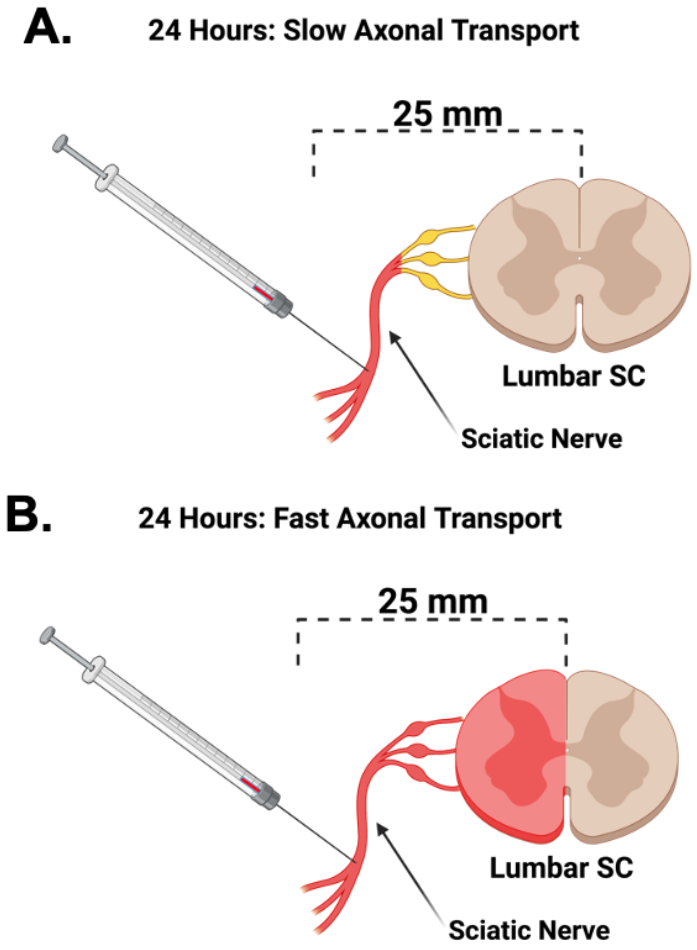
Ayers et al., 2009; Langenfeld et al., 2016

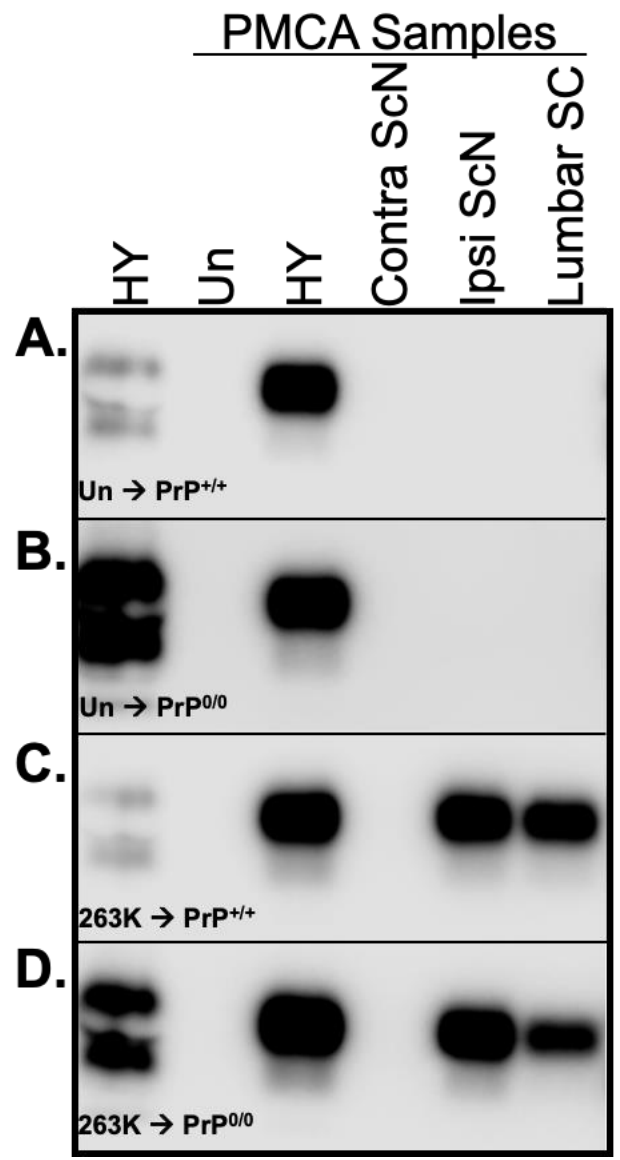
Sam Koshy

Prion pathogenesis

Sciatic nerve inoculation

- Previous studies have measured rates of spread.
- Can we directly measure the inoculum PrP^{Sc} velocity?
- Utilize prion replication deficient systems.
    - Strain
    - Host

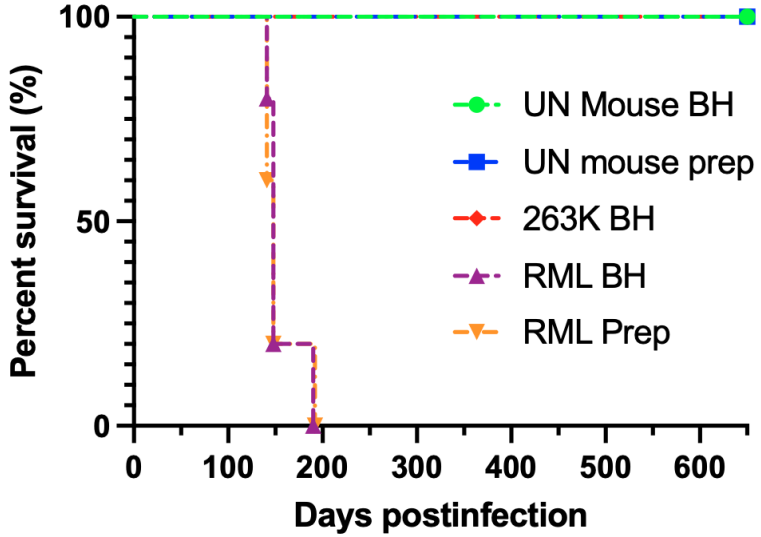


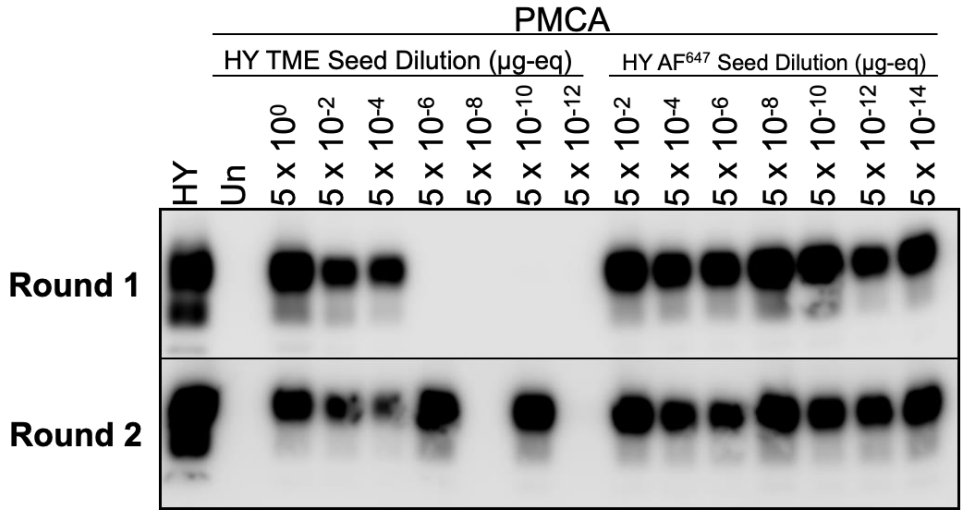


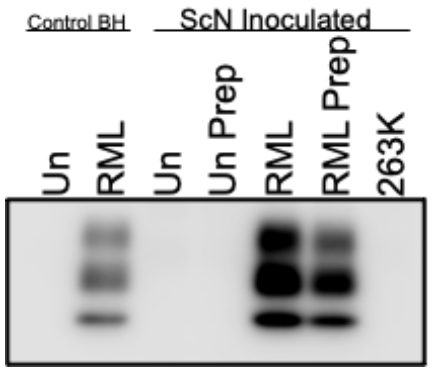
Prion pathogenesis

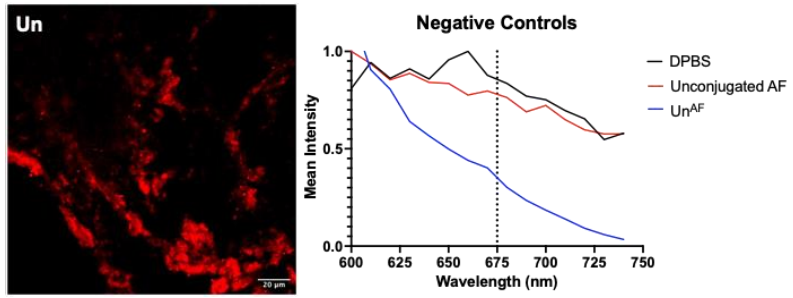
Sciatic nerve inoculation

Brain homogenate → Wenborn Prep → AF^{647} → Analysis





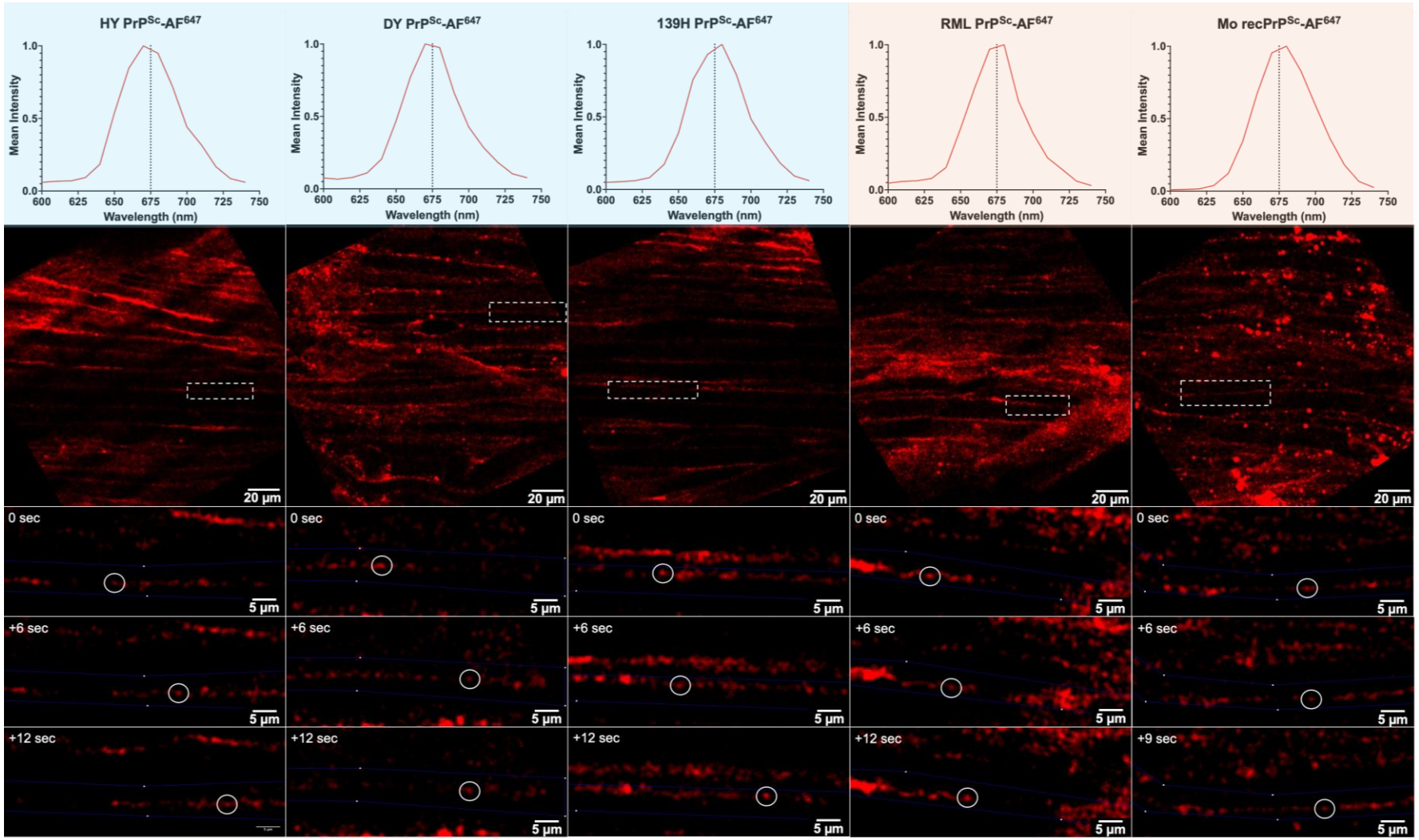




- Imaging PrP^{Sc} in sciatic nerves of live animals.

Prion pathogenesis

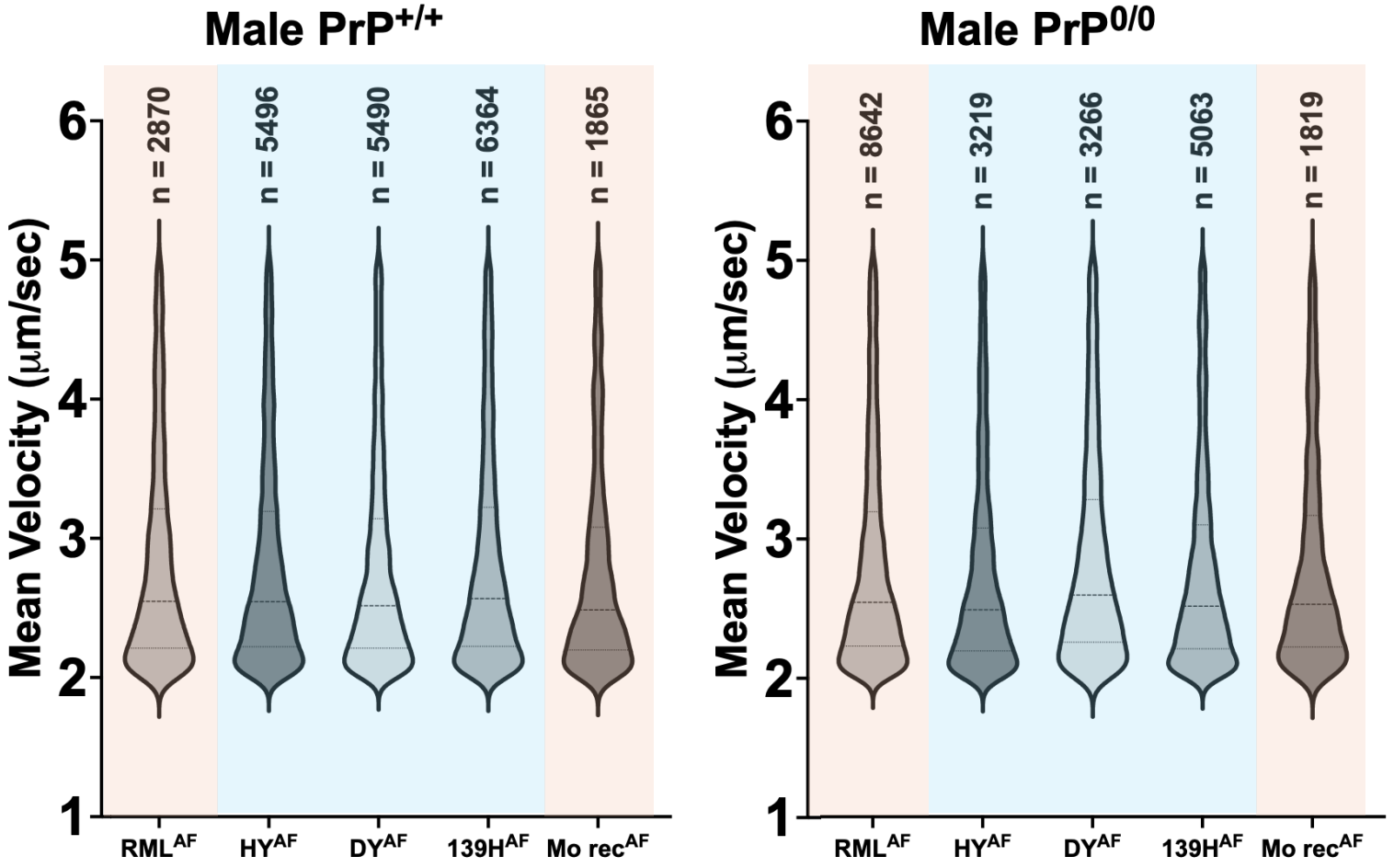
Sciatic nerve inoculation



- Imaging PrP^{Sc} in sciatic nerves of live animals.

Prion pathogenesis

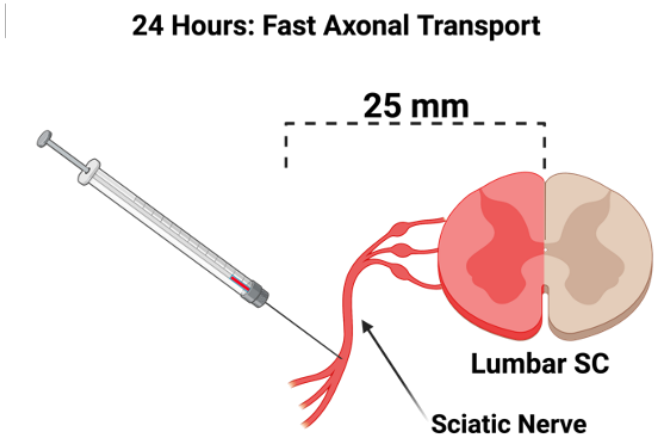
Sciatic nerve inoculation

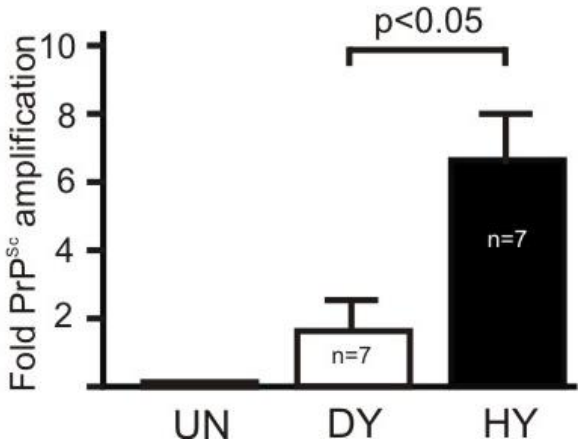


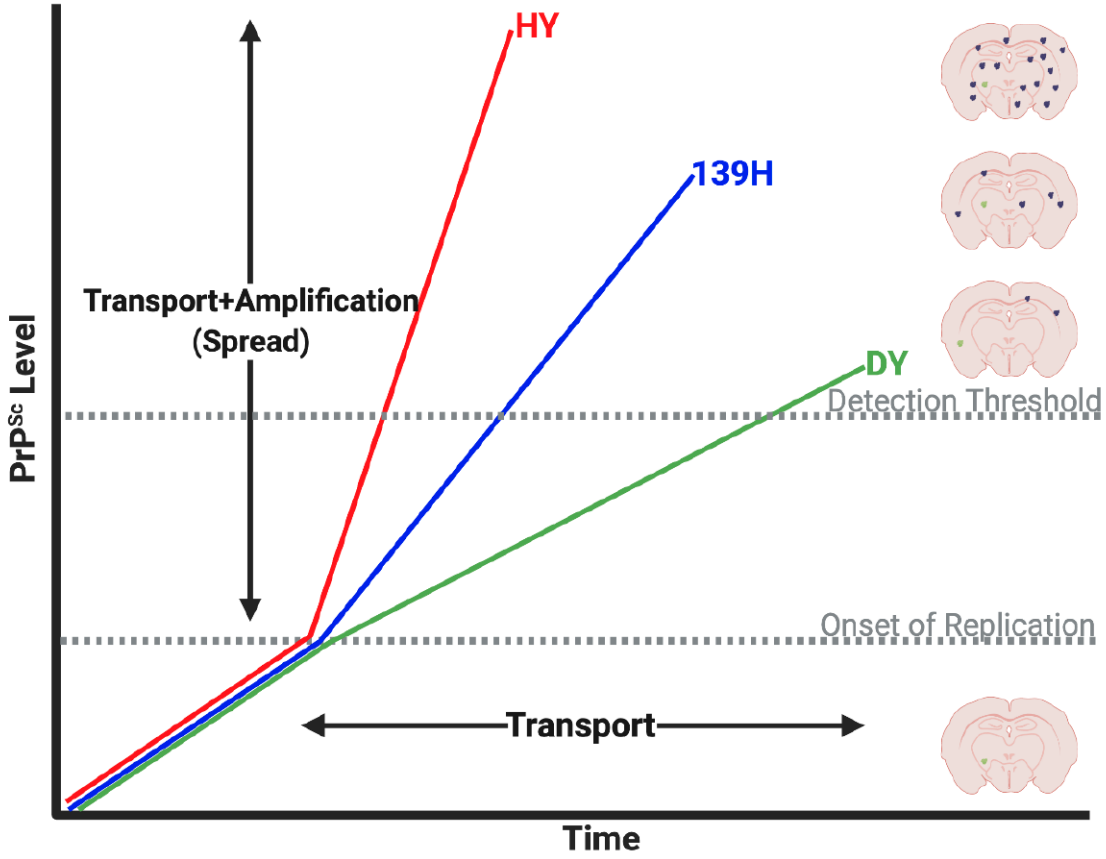
- PrP^{Sc} velocities are consistent with fast axonal transport
- Strain specific velocities are not observed
- Not dependent on expression of PrP^{C}

Prion pathogenesis

Sciatic nerve inoculation





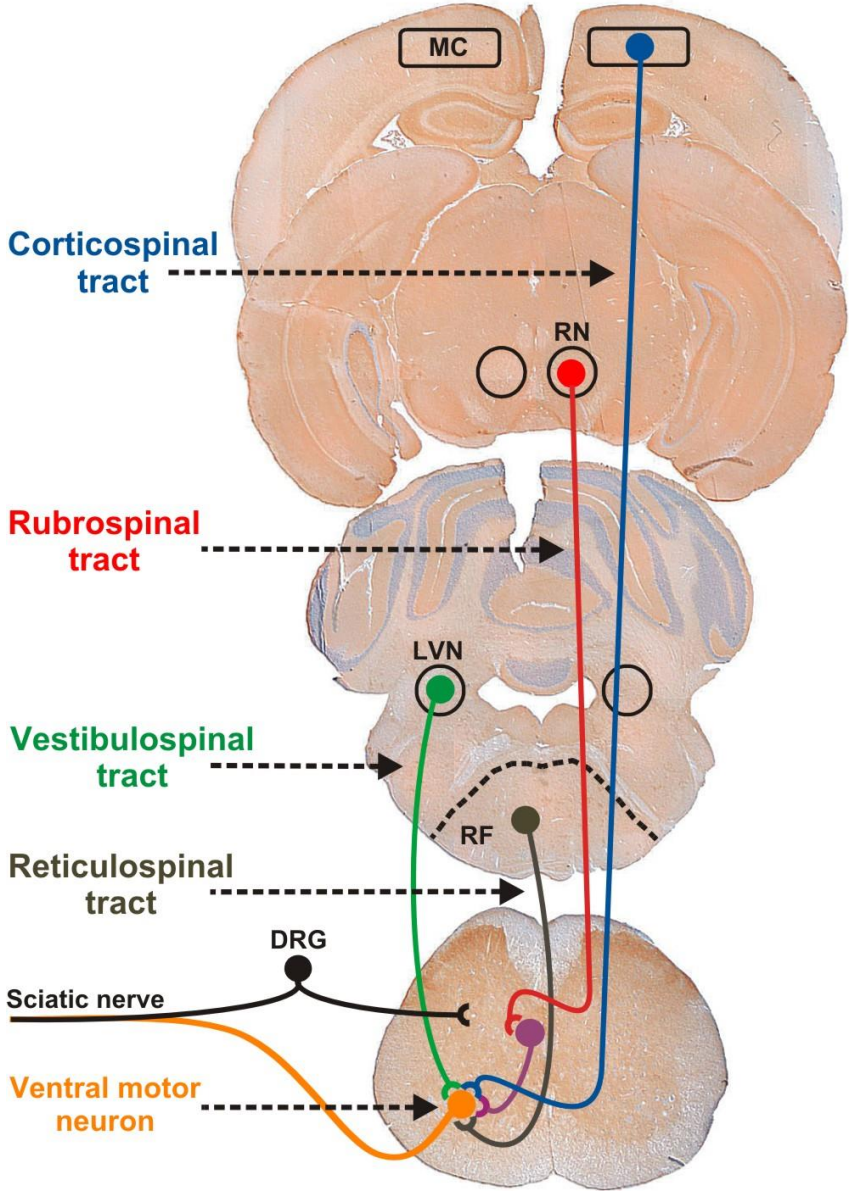


- Decoupling prion replication vs. prion transport

Prion pathogenesis

Summary

- PrP^{Sc} travels along known neuroanatomical pathways
- Transport in the nervous system is a shared feature between prion and prion-like diseases
- Patterns of spread are independent of prion strain.
  - Percentage of inc. period vs. dpi
- PrP^{Sc} velocity in the sciatic nerve is consistent with fast axonal transport
  - Independent of prion strain or PrP^{C}
- Sciatic nerve inoculation is a useful method to study other aspects of prion biology.
  - Prion strain interference



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Tess Gunnels
Jay Hrdlicka
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MINNESOTA CENTER FOR PRION RESEARCH AND OUTREACH
UNIVERSITY OF MINNESOTA
Driven to Discover℠

CIDRAP

North American interdisciplinary chronic wasting disease research consortium
- United States Department of Agriculture NC1209 -

DEPARTMENT OF NATURAL RESOURCES DNR MICHIGAN

NIH National Institutes of Health

CJD FOUNDATION

CREUTZFELDT-JAKOB DISEASE FOUNDATION, INC.
Supporting Families Affected by Prion Disease

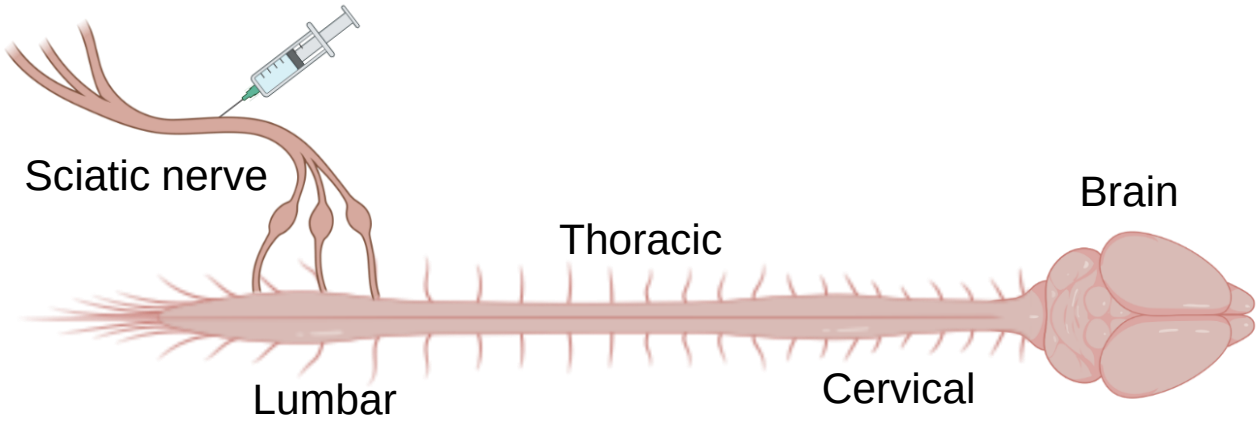
Prion Research Center
Established 2011
Colorado State University

Prion pathogenesis
Summary

- PrP^{Sc} travels along known neuroanatomical pathways
- Transport in the nervous system is a shared feature between prion and prion-like diseases
- Patterns of spread are independent of prion strain
    - Percentage of inc. period vs. dpi
- PrP^{Sc} velocity in the sciatic nerve is consistent with fast axonal transport
- Targeting prions to the same population of neurons is a powerful means to investigate prion evolution.

Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution



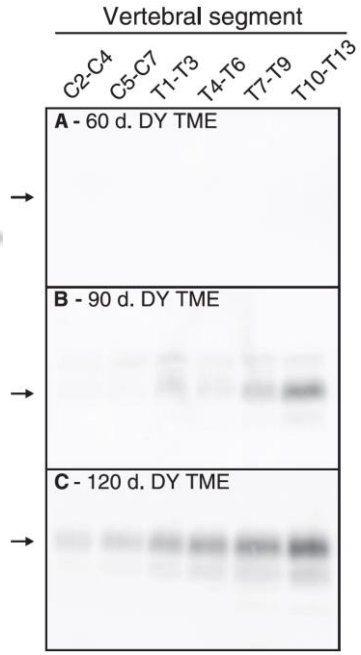
DY — 217 d. → **DY**

HY — 78 d. → **HY**

DY — 60 d. → **HY** — ?? → ??

DY — 90 d. → **HY** — ?? → ??

DY — 120 d. → **HY** — ?? → ??



Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution

First Inoculation	Interval between Inoculations	Second Inoculation	Clinical Signs	Onset of clinical symptoms: After 1st Inoculation	Onset of clinical symptoms: After 2nd Inoculation
DY TME	120 days	Mock	DY TME	217±2[a]	n.a.
Mock	120 days	HY TME	HY TME	n.a.	78±2
DY TME	60 days	HY TME	HY TME	138±3	78±3[b]
DY TME	90 days	HY TME	HY TME	180±7	90±7[c]
DY TME	120 days	HY TME	DY TME	220±3[d]	100±3

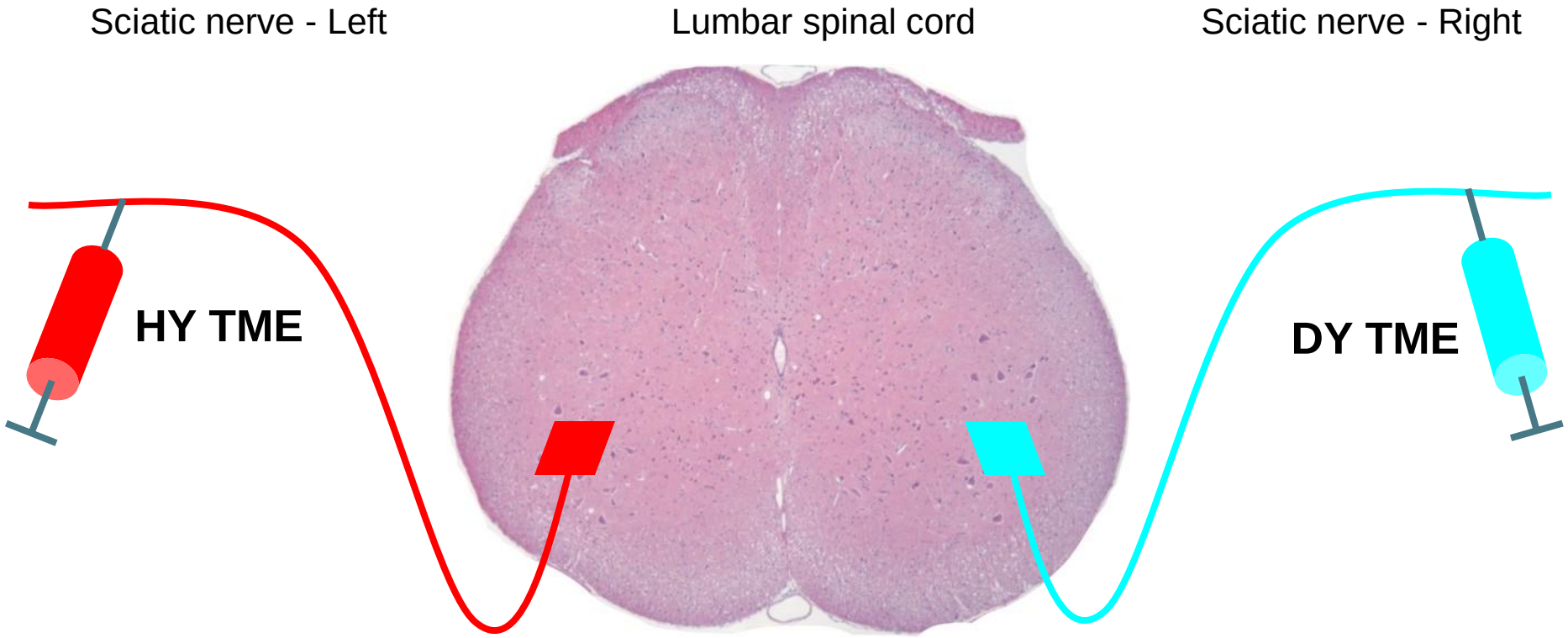
- DY TME can interfere with the emergence of HY TME

Bartz et al., 2007, *Journal of Virology*

Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution

If DY TME agent replication, ipsilateral to the site of inoculation is responsible for diminishing the ability of the HY TME agent to cause disease, then inoculation of the HY TME agent in the sciatic nerve contralateral to DY TME agent inoculation would result in animals succumbing to HY TME with incubation periods similar to animals inoculated with the HY TME agent alone.



Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution

First Inoculation (Right s.n.)	Interval between Inoculations	Second Inoculation (Left s.n.)	Clinical Signs	PrP^{Sc} migration	A/I[a]	Onset of clinical symptoms: After 1st Inoculation	Onset of clinical symptoms: After 2nd Inoculation
Mock	90 days	HY TME	HY TME	21 kDa	5/5	n.a.	77±3[b]
DY TME	90 days	HY TME	HY TME	21 kDa	5/5	167±3	77±3[c]
DY TME	90 days	Mock	DY TME	19 kDa	5/5	229±3	n.a.
Mock	120 days	HY TME	HY TME	21 kDa	5/5	n.a.	73±3
DY TME	120 days	HY TME	HY TME	21 kDa	5/5	197±11	77±11[c]
DY TME	120 days	Mock	DY TME	19 kDa	4/4	232±4	n.a.

[a] Number affected / number inoculated
[b] Average days postinfection ± standard deviation
[c] Incubation period similar compared to animals inoculated with the HY TME agent alone ($P>0.05$)
n.a. – not applicable
s.n. – sciatic nerve

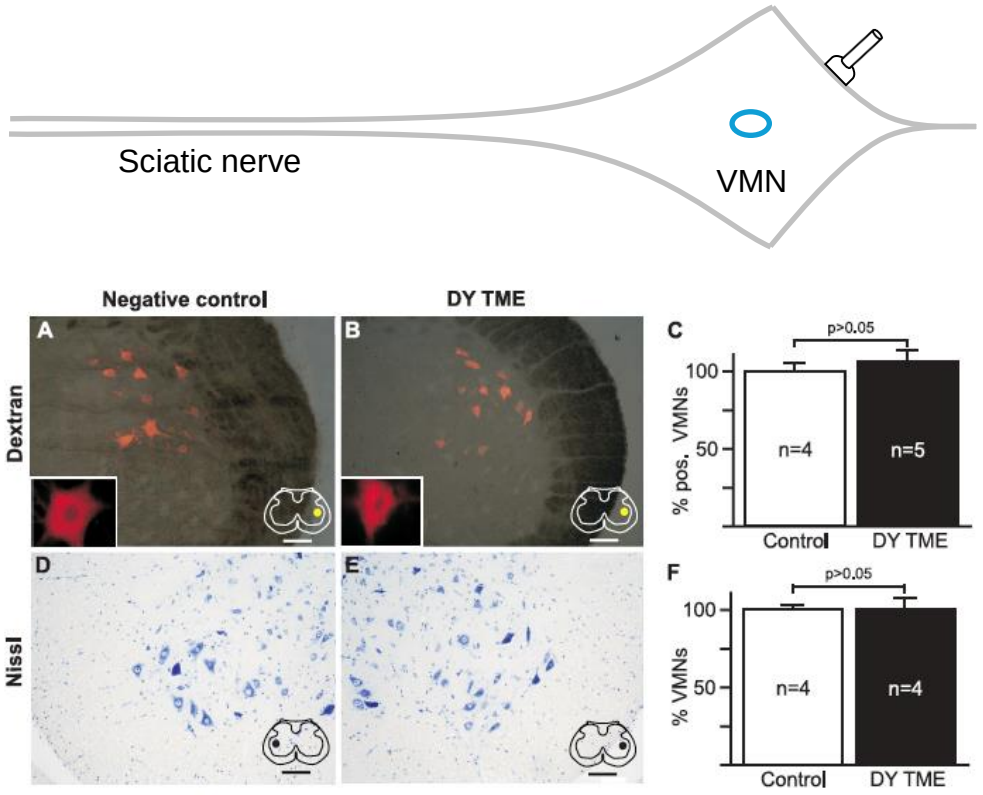
- Prion strain interference is in VMNs

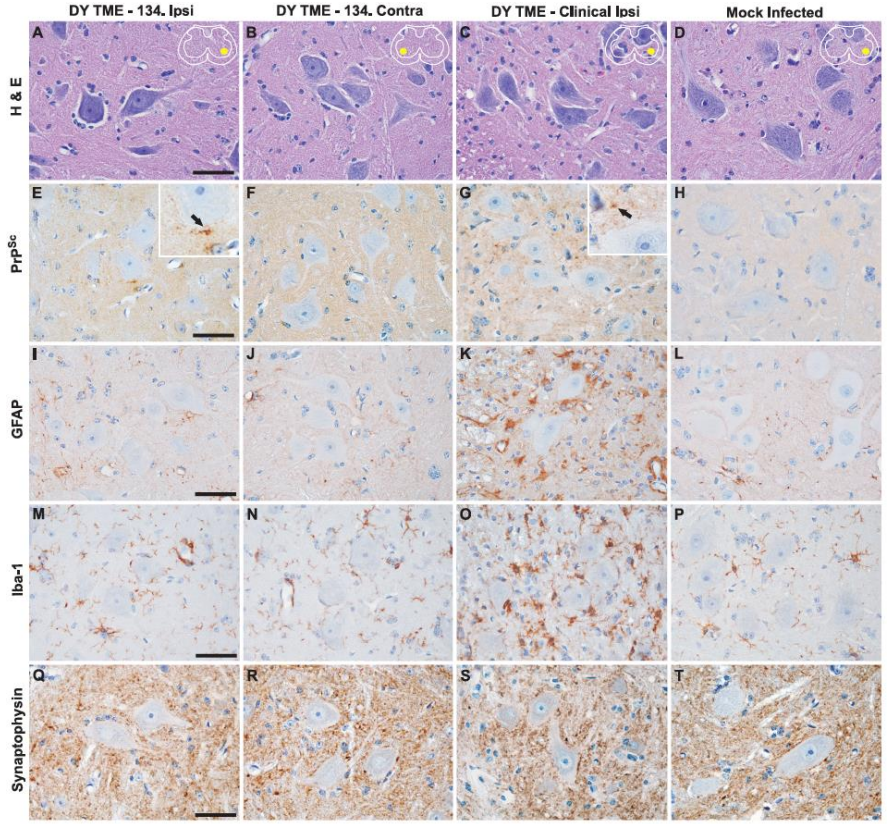
Bartz et al., 2007, *Journal of Virology*

Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution

- How can DY TME prevent HY TME from causing disease?



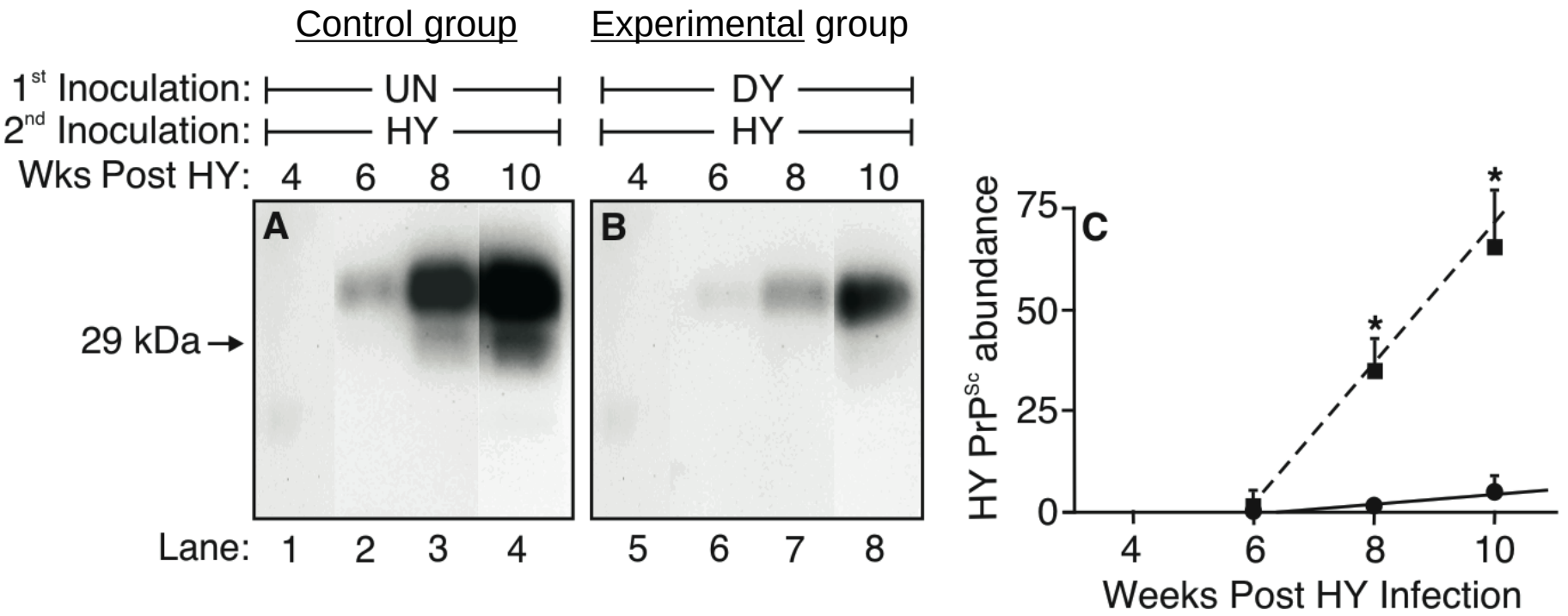


- The only detectable change is the deposition of DY PrP^{Sc}

Bartz et al., 2007; Shikiya et al., 2010 *Journal of Virology*

Prion pathogenesis

Sciatic nerve inoculation – Prion strain evolution



- The dominant strain suppresses but does not eliminate replication of the minor strain

Bartz et al., 2007, *Journal of Virology*

Prion pathogenesis

Prion strain evolution

Coinfecting Prion Strains Compete for a Limiting Cellular Resource[▽†]

Ronald A. Shikiya,[1] Jacob I. Ayers,[1] Charles R. Schutt,[1] Anthony E. Kincaid,[1,2] and Jason C. Bartz[1*]

Prion Interference Is Due to a Reduction in Strain-Specific PrP^{Sc} Levels[▽]

Jason C. Bartz,[1*] Michelle L. Kramer,[1] Meghan H. Sheehan,[1] Jessica A. L. Hutter,[1] Jacob I. Ayers,[1] Richard A. Bessen,[3] and Anthony E. Kincaid[2]

Coinfecting Prion Strains Compete for a Limiting Cellular Resource[▽†]

Ronald A. Shikiya,[1] Jacob I. Ayers,[1] Charles R. Schutt,[1] Anthony E. Kincaid,[1,2] and Jason C. Bartz[1*]

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Incongruity between Prion Conversion and Incubation Period following Coinfection

Katie A. Langenfeld,[a] Ronald A. Shikiya,[a] Anthony E. Kincaid,[a,b] Jason C. Bartz[a]

	PMCA strain interference							
PMCA round	500 µg eq. HY TME	500 µg eq. DY TME	Mock	500 µg eq. HY TME + 500 µg eq. DY TME	50 µg eq. HY TME + 500 µg eq. DY TME	5 µg eq. HY TME + 500 µg eq. DY TME	$5x10^{-1}$ µg eq. HY TME + 500 µg eq. DY TME	$5x10^{-2}$ µg eq. HY TME + 500 µg eq. DY TME
1	HY	DY	-	HY/DY	DY	DY	DY	DY
2	HY	DY	-	HY	HY	DY	DY	DY
3	HY	DY	-	HY	HY	DY	DY	DY
4	HY	DY	-	HY	HY	HY	HY	DY
5	HY	DY	-	HY	HY	HY	HY	DY
6	HY	DY	-	n.d	n.d	n.d	n.d	DY
7	HY	DY	-	n.d	n.d	n.d	n.d	DY
8	HY	DY	-	n.d	n.d	n.d	n.d	DY
9	HY	DY	-	n.d	n.d	n.d	n.d	HY
10	HY	DY	-	n.d	n.d	n.d	n.d	HY

1. Strain interference is governed by the relative onset of prion replication between the strains in a common population of cells.
2. Prion strains compete for PrP^{C}.
3. The blocking strain can suppress replication, but does not eliminate, the superinfecting strain.

Protein Misfolding Cyclic Amplification

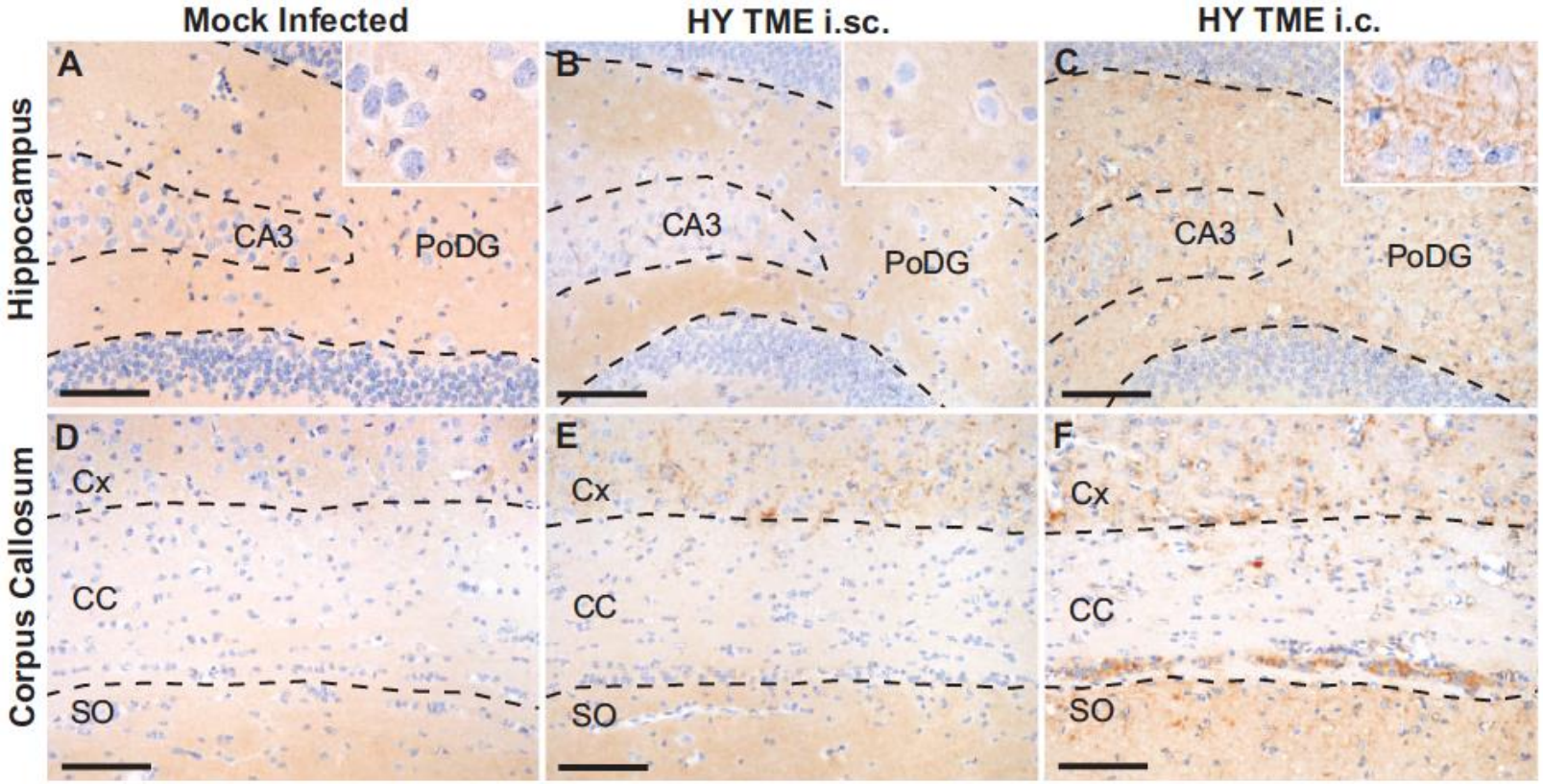
Generates infectious prions

Dilution	Result for indicated inoculum[a]			
	PMCA generated		Brain derived	
	Incubation period (days ± SEM)	No. of hamsters affected/total no. inoculated	Incubation period	No. of hamsters affected/total no. inoculated
10^{-2}	83 ± 3[b]	5/5	61 ± 3	5/5
10^{-3}	93 ± 3	5/5	71 ± 3	5/5
10^{-4}	99 ± 4	5/5	79 ± 9	5/5
10^{-5}	164 ± 111	5/5	89 ± 6	5/5
10^{-6}	186 ± 24	5/5	98 ± 2[b]	5/5
10^{-7}	214 ± 11	3/5	134 ± 9[b]	4/5
10^{-8}	>400	0/5	192 ± 54[b]	3/5
10^{-9}	>400	0/5	>400[b]	0/5
None (mock inoculation)	>400	0/5	>400	0/5
	$10^{8.6}$ LD_{50}		$10^{9.3}$ LD_{50}	

- *In vitro* PMCA generation of high titer HY TME agent.

Shikiya & Bartz, 2011

HY PrP^{Sc} deposition in hippocampus & corpus callosum



- Following i.c. inoculation, HY PrP^{Sc} is detected in the hippocampus and corpus collosum.
- Lack of PrP^{Sc} deposition is not due to a lack of ability to replicate in hippocampus.

Ayers et al., 2009 Journal of Virology

Temporal and spatial spread of HY and DY PrP^{Sc}:

Deposition at clinical disease

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	+

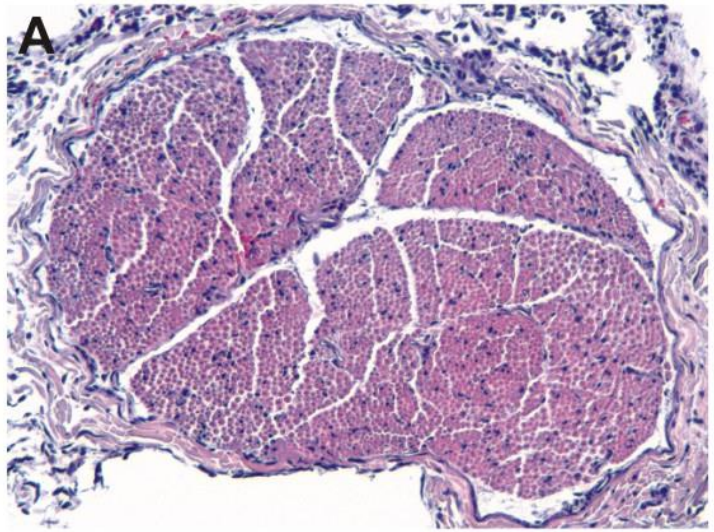
[a] +, present; 0, absent.

- PrP^{Sc} deposition is more widespread in DY TME infected hamsters compared to HY TME infected hamsters.

Ayers et al., 2009 Journal of Virology

What is the composition of the sciatic nerve?

- Both sensory, motor, and sympathetic axons
  - ~ 6% motor
  - ~ 71% sensory
  - ~ 23% sympathetic

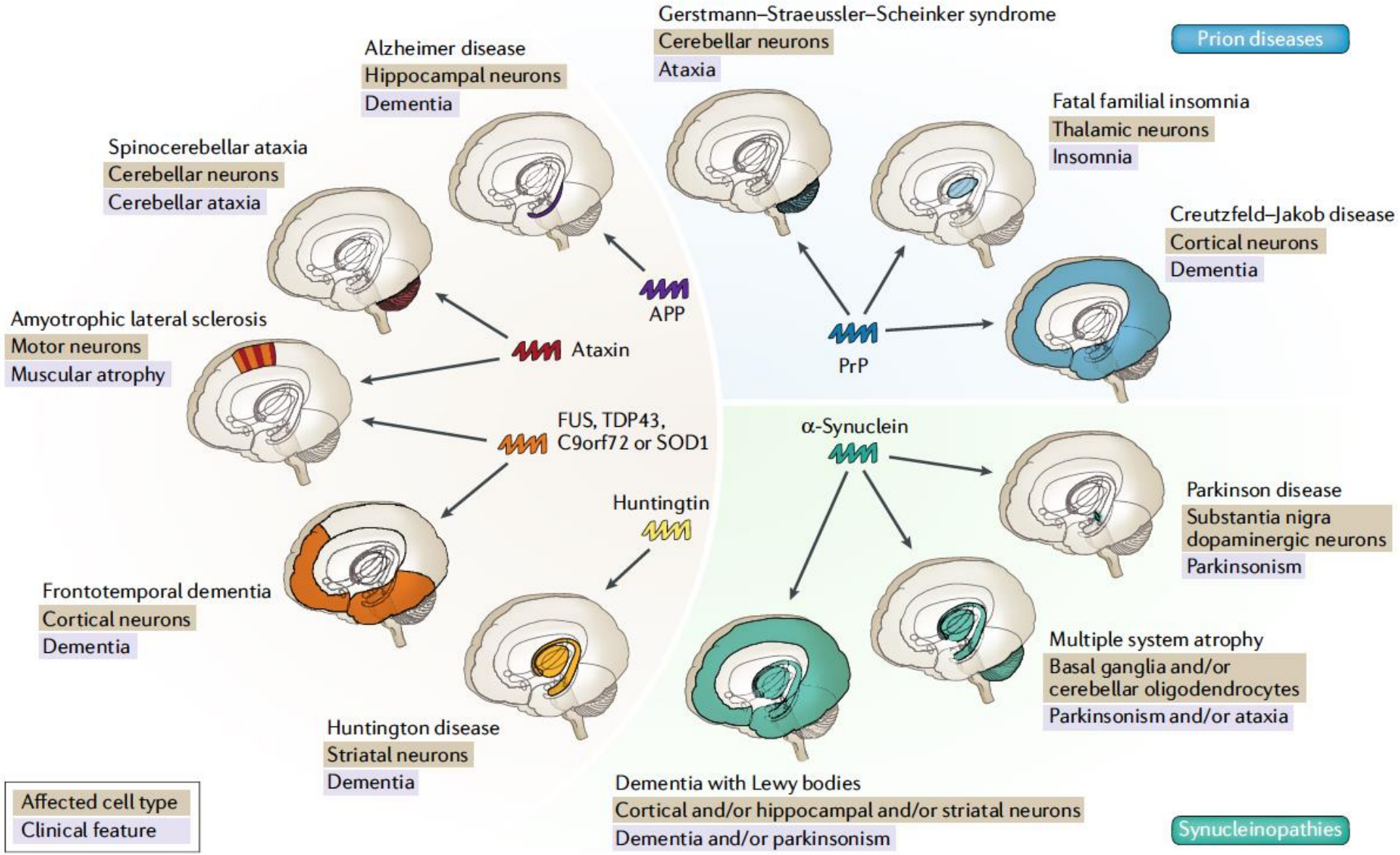


- Inoculation in this nerve should present the agent to all fiber types.

- Inject dextran into sciatic nerve

Prion and prion-like diseases

Prion conversion



Schekel & Aguzzi, 2018