FULL PAPER *Anatomy*

Localization of Sympathetic, Parasympathetic and Sensory Neurons Innervating the Distal Ileum of the Cattle

Yasushige OHMORI[1)]*, Yasuro ATOJI[2)], Shouichiro SAITO[2)], Hiroshi UENO[3)], Yasuo INOSHIMA[4)] and Naotaka ISHIGURO[4)]

[1)]*Laboratory of Animal Morphology and Function, Graduate School of Bioagricultural Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464–8601 and* [2)]*Laboratories of Veterinary Anatomy,* [3)]*Veterinary Clinical Radiology and* [4)]*Food and Environmental Hygiene, Faculty of Applied Biological Sciences, Gifu University, Yanagido 1–1, Gifu 501–1193, Japan*

(Received 30 April 2008/Accepted 24 July 2008)

ABSTRACT. After oral challenge of the pathological prion protein, the causative agent of bovine spongiform encephalopathy, the pathogen was first detected in the distal ileum and then deposited in the brain. The present study aims determining the possible neuronal transporting pathways from the ileum to the brain in the cattle using a tracer protein. After horseradish peroxidase was injected into the wall of the distal ileum in the calf, almost all labeled neurons were detected in the celiac and cranial mesenteric ganglion complex. Only a few labeled neurons existed in the caudal mesenteric ganglion and the paravertebral ganglia. They were sympathetic postganglionic neurons. In the dorsal root ganglia T5 to L4, some sensory neurons were found to be labeled. Only a small number of parasympathetic preganglionic neurons were labeled in the dorsal motor nucleus of the vagus nerve. No labeled sensory neurons were found in the nodose ganglion. These results suggest that the pathological prion protein is mainly transported to the spinal cord and brain via the sympathetic nervous system and partially via the sensory neurons in the dorsal root ganglia. The vagus nerve does not seem to contribute to the transport of the pathogen from the ileum directly.

KEY WORDS: autonomic nervous system, cattle, extrinsic innervation, ileum, retrograde labeling.

J. Vet. Med. Sci. 70(12): 1289–1294, 2008

Transmissible spongiform encephalopathies, such as Creutzfeldt-Jakob disease in the human, bovine spongiform encephalopathy (BSE) in the cattle and scrapie in the sheep, are infectious and invariably fatal neurodegenerative disorders of the central nervous system [4, 7, 21]. The causative agent of transmissible spongiform encephalopathies is an abnormal conformer of cellular prion protein, which is called as prion or PrP^{Sc} [13]. The feeding of contaminated meat and bone meal was recognized as the main mode of transmission of BSE [6]. Experimental time-course studies on the pathogenesis of PrP^{Sc} in rodents strongly suggested that infection spread from the gastrointestinal tract via the splanchnic and vagus nerves to the spinal cord and brain after an intraperitoneal, intragastric or oral administration of PrP^{Sc} [2, 3, 15, 22, 23]. Recently, the same result was demonstrated in the cattle after an oral challenge of PrP^{Sc} [12].

The origins of the sympathetic and parasympathetic innervation to the small intestine were mainly studied using retrograde axonal transport of tracers. The sympathetic supply to the ileum in the rat arose primarily from postganglionic neurons in the celiac and cranial mesenteric ganglion complex (C/CrMG) and paravertebral ganglia (PVGs) [20]. After injection of horseradish peroxidase (HRP) into the small intestine of the cat [5], dog [14] and monkey [29], labeled sensory neurons were found in the dorsal root ganglia (DRGs) and/or nodose (vagal distal) ganglion (NG). Parasympathetic preganglionic neurons innervating the small intestine of the rat [1, 33] and cat [26] were located in the dorsal motor nucleus of the vagus nerve (DMNV).

The distal ileum is one of the most possible sites on prion neuroinvasion, because it is an early detection site of PrP^{Sc} accumulation following the oral prion challenge in the cattle [30]. Via sympathetic, parasympathetic and sensory nerves, PrP^{Sc} may be transported from the ileum to the spinal cord and brain. However, no studies using retrograde HRP labeling have been carried out in the ileum of the cattle. The present study aims determining the location, number and segmental distribution of sympathetic, parasympathetic and sensory neurons innervating the distal ileum of the cattle using retrograde axonal transport of HRP. The data obtained in the present study may reveal the autonomic pathways via which PrP^{Sc} is potentially transported from the ileum to the spinal cord and brain.

MATERIALS AND METHODS

Three male Holstein calves aged two months (weighing about 80 kg) were purchased from a local dairy farm. Food and water were available *ad libitum*. The cattle were treated in accordance with the Guidelines for Care and Use of Experimental Animals in the Gifu University. A special effort was made to minimize suffering and the number of animals used.

Under heavy sedation with xylazine hydrochloride (0.2 mg/kg, i.v.) and local anesthesia with 2% lidocaine hydrochloride (30 m*l*, s.c.), an incision (about 15 cm long) was made in the paralumbar fossa of the right lateral abdominal region. The ileum with the ileocecal fold was exposed

* CORRESPONDENCE TO: OHMORI, Y., Laboratory of Animal Morphology and Function, Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya 464–8601, Japan.
e-mail: ohmori@agr.nagoya-u.ac.jp

through this incision. Using a 10 μl-microsyringe with a 30-gauge needle, 100 or 200 μl of a mixed saline solution of 40% HRP (grade I, Roche Diagnostics, Indianapolis, IN, U.S.A.), 4% wheat germ agglutinin-conjugated HRP (Toyobo, Osaka, Japan) and 0.15% cholera toxin subunit B-conjugated HRP (List Biological Laboratories, Campbell, CA, U.S.A.) (this mixture shall be described as HRP hereafter) was injected into 10 or 20 points of the antimesenteric wall in a segment of the ileum between 10 and 20 cm proximal from the ileocecal junction. After closing the abdominal wall, atipamezol (0.02 mg/kg, i.v.) was injected for the recovery from sedation.

After a survival time of 4 or 7 days, the calves were heavily sedated with xylazine and purfused through the right common carotid artery with saline (30 *l*), followed by a fixative solution (50 *l*) consisting of 1.25% glutaraldehyde and 1% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Finally, the cattle were perfused with 10% sucrose in phosphate buffer (20 *l*). Bilateral C/CrMG, caudal mesenteric ganglion (CaMG), PVGs and DRGs from thoracic segment (T) 4 to sacral segment (S) 1, NG and the medulla oblongata about 2.5 cm in length were dissected out, soaked at least overnight in phosphate buffered 30% sucrose at 4°C and then embedded in Tissue-Tec II O.C.T. Compound. Serial tissue sections 60 μm thick of C/CrMG, CaMG, PVGs, DRGs and NG were cut in the longitudinal plane with a cryostat, mounted on glass slides coated with chrome alum-gelatin and then processed for HRP with tetramethylbenzidine as chromogen [24]. Medulla oblongata sections 60 μm of thickness were cut in the transverse plane, processed for HRP and then mounted on gelatin-coated glass slides. All sections were counterstained with 1% neutral red solution. The injection sites of HRP were not observed histologically.

Every section through the C/CrMG, CaMG, PVGs, DRGs, NG and medulla oblongata was examined under a light microscope (BH-2, Olympus, Tokyo, Japan) for the counting of HRP-labeled neurons. The number of labeled neurons was not corrected for double counting except for the omission of obvious fragments.

RESULTS

After injections of HRP into the ileum, the number of retrogradely labeled neurons at each site in the three calves is given in Table 1. The total number of labeled neurons for the three calves was 709, 5,126 and 3,596 cells, respectively. These findings are described in detail below.

Sympathetic nervous system

Postganglionic neurons: Almost all labeled neurons were found in the C/CrMG (Table 1). Labeled neurons were polygonal or oval in shape (Fig. 1A). Many of labeled neurons were relatively smaller in the cell body size and densely distributed in the caudoventral area of the C/CrMG. Larger labeled neurons were scattered in the whole of the ganglion. In the CaMG and PVGs, only a small number of postganglionic neurons were found to be labeled (Table 1). The labeled neurons were polygonal in shape (Fig. 1B, C). In the PVGs, they were mainly distributed in T13 to lumbar segment (L) 2 on both sides.

Sensory neurons: Some labeled neurons were observed in the left and right DRGs (Table 1). They were distributed in the DRGs T5 to L3 (right side) or L4 (left side) (Fig. 2). More than 80% of labeled DRG neurons were located in the DRGs T9 to L1 (highest in T12). Labeled neurons were round or oval in shape and smaller in the cell body size than unlabeled ones (Fig. 1D). They scattered throughout the individual ganglion at all levels.

Parasympathetic nervous system

Preganglionic neurons: The left and right DMNVs in the medulla oblongata of two cases contained extremely a small number of labeled neurons (Table 1). They were scattered from 2.5 mm caudal to 1.5 mm cranial to the obex. Labeled neurons were triangular or fusiform in shape (Fig. 1E) and distributed in all areas of the DMNV on the transverse plane.

Sensory neurons: Sensory neurons in the NG were found to be scattered between preganglionic nerve fibers (Fig. 1F). No labeled neurons were detected in the NG on the both sides (Table 1).

DISCUSSION

The Peyer's patches in the distal ileum of the cattle were one of the most possible sites on PrP^{Sc} neuroinvasion [30]. As the Peyer's patches are localized in the antimesenteric wall of the ileum, HRP was injected into the intestinal wall in the antimesenteric side of the distal ileum.

In the cattle No. 1, the total volume of injected HRP was smaller and the post-injection survival time was shorter than those in the others. The number of labeled sympathetic postganglionic neurons in the C/CrMG was increased when the total volume of HRP and the survival time were simulta-

Table 1. The number of labeled neurons in the celiac and cranial mesenteric ganglion complex (C/CrMG), caudal mesenteric ganglion (CaMG), paravertebral ganglia (PVGs), dorsal root ganglia (DRGs), dorsal motor nucleus of the vagus nerve (DMNV) and nodose ganglion (NG) following HRP injection into the distal ileum of the three calves

Cattle no.	Volume of HRP (μl)	Survival time (day)	Sympathetic nervous system				Parasympathetic nervous system	
			Postganglionic			Sensory	Preganglionic	Sensory
			C/CrMG	CaMG	PVGs	DRGs	DMNV	NG
1	100	4	519	0	1	189	0	0
2	200	7	4,846	24	27	215	14	0
3	200	7	3,493	9	1	82	11	0

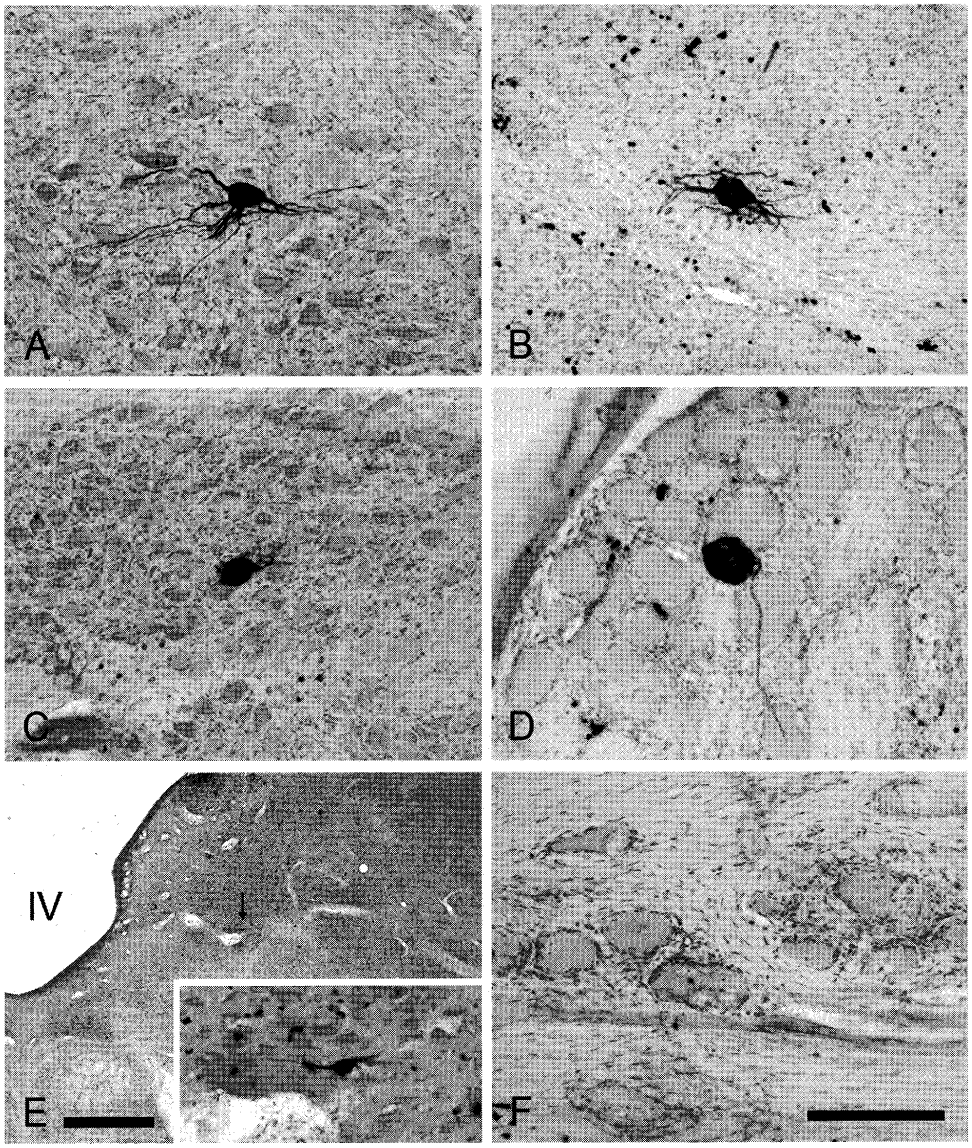

Fig. 1. Retrogradely labeled neurons in the celiac and cranial mesenteric ganglion complex (A), caudal mesenteric ganglion (B), right sympathetic trunk ganglion T13 (C), left dorsal root ganglion T13 (D), right dorsal motor nucleus of the vagus nerve (E) and left nodose ganglion (F: no labeled neurons) after injection of HRP into the distal ileum of the cattle. Arrow: labeled parasympathetic preganglionic neuron; IV: fourth ventricle. Bar in F = 100 μm for A-D, F, and insertion in E. Bar in E = 1 mm.

neously increased. On the other hand, the number of labeled sensory neurons in the DRGs of the cattle No. 1 was intermediate to those in the cattle Nos. 2 and 3. There may be some differences in the rate of HRP transport between sympathetic postganglionic neurons and sensory ones. In the rat, the rate of the retrograde transport of nerve growth factor is faster in the sensory neurons than in the sympathetic postganglionic neurons [27]. The amount of HRP in the cell body is noticeably reduced as early as 3 days after initial arrival of HRP by the lysosomes containing the hydrolytic enzyme. Between 4–8 days, most lysosomes are depleted of HRP [19, 25, 32]. Therefore, the survival time of 7 days appears optimal regarding HRP labeling of neurons in the calf.

The intestine is innervated by sympathetic and parasympathetic nervous systems (see [9] for review). These two systems control intestinal functions in antagonism with each other. Using retrograde axonal transport of tracers, it is known that the small intestine is innervated by four kinds of extrinsic neurons (Fig. 3A).

In the ileum of the rat, the primary source (65%) of sympathetic postganglionic fibers was C/CrMG and 30% of labeled neurons were located in the PVGs T9 to T11 and splanchnic ganglia [20]. In the present study, almost all labeled neurons were sympathetic postganglionic. Most of them were located in the C/CrMG and only a few in the CaMG and PVGs. This may be a characteristic of sympathetic innervation in the cattle ileum.

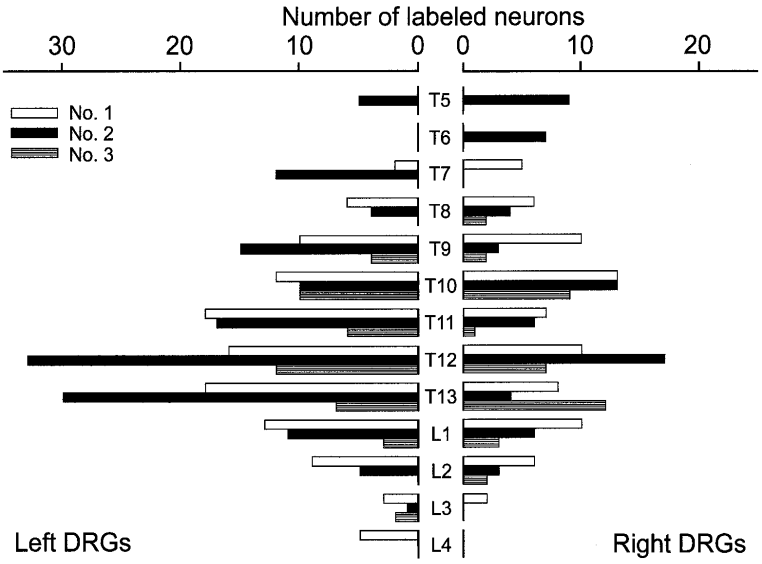

Fig. 2. Segmental distribution of HRP-labeled sensory neurons in the dorsal root ganglia (DRGs) on each side. White bars indicate labeling in the cattle No. 1, black bars in No. 2 and hatched bars in No. 3.

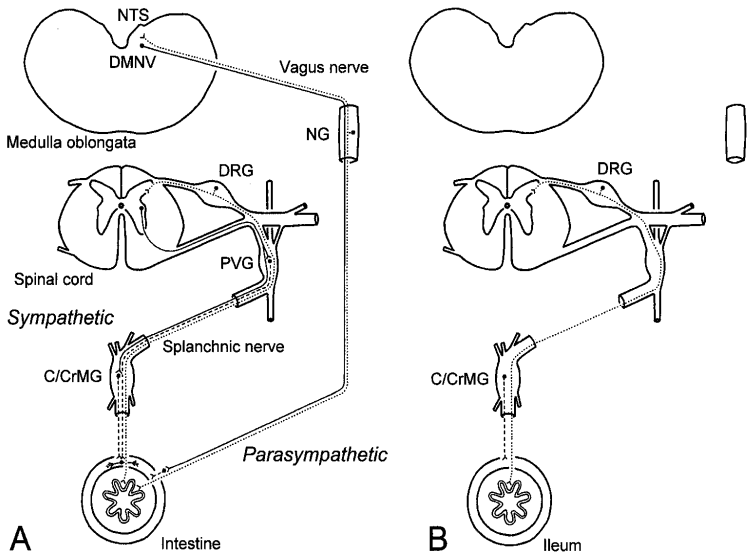

Fig. 3. Schematic representation for the extrinsic autonomic innervation to the small intestine of the typical mammals (A) and to the distal ileum of the calf (B). The extrinsic innervation consists of sympathetic, parasympathetic and sensory components. The possible transporting pathways of pathological prion protein from the ileum to the spinal cord and brain in the cattle are mainly constructed by sympathetic postganglionic neurons in the celiac and cranial mesenteric ganglion complex (C/CrMG) and partially by sympathetic sensory neurons in the dorsal root ganglion (DRG). The vagus nerve does not contribute to the transport from the ileum directly. DMNV, dorsal motor nucleus of the vagus nerve; NG, nodose ganglion; NTS, nucleus of the solitary tract; PVG, paravertebral ganglion; Solid line: preganglionic fibers; Dashed line: postganglionic fibers; Dotted line: sensory fibers.

The small intestine was, furthermore, innervated by sympathetic sensory neurons in the DRGs of the cat (duodenum [5]), sheep (ileum [8]), dog (duodenum [14]) and monkey (duodenum, jejunum and ileum [29]). In the ileum of the sheep, the labeled sensory neurons were located in the DRGs T5 to L4 (highest in T13 to L3) [8]. On the other hand, those in the ileum of the monkey were existed in DRGs T4 to L1 (highest in T8 and T9) [29]. In the present

study, some labeled neurons were sympathetic sensory neurons in the DRGs T5 to L4 (highest in T12). There are some differences among animals in the segmental distribution pattern of sensory neurons. These neurons are the second group of extrinsic innervation to the ileum.

Parasympathetic preganglionic neurons in the DMNV projected to the duodenum, jejunum and ileum in the rat [1, 33] and cat [26]. However, the number of labeled neurons after injections into the small intestine was much smaller than that after stomach injections [1, 26, 33]. In the rat stomach, parasympathetic preganglionic neurons formed direct synaptic contacts on myenteric ganglion neurons [10]. The density of the parasympathetic preganglionic fibers in the myenteric ganglion of the rat small intestine declined sharply below the antral sphincter [16]. In the present study, only a few labeled neurons were found in the DMNV of the cattle Nos. 2 and 3. Although the stomach receives a great number of parasympathetic preganglionic projections from the DMNV, the ileum may receive extremely the small number of vagal preganglionic projections directly.

Labeled parasympathetic sensory neurons were found in the NG after injections into the duodenum of the cat [5] and dog [14]. The number of labeled neurons in the NG of the cat and dog was larger than that in the DRGs after duodenal injections, but there are no data for the ileum. In the present study, no labeled neurons were found in the NG. There may be some differences in the parasympathetic sensory innervation between the portions of the small intestine or species.

In addition to four kinds of neurons mentioned above, intestinofugal neurons have been reported [17, 18, 28, 31]. They have cell bodies in the gut wall and send their axons to prevertebral ganglia, where they form synapses with sympathetic postganglionic neurons. Although no axon terminals labeled by HRP were detected in the C/CrMG and CaMG of the present study, intestinofugal neurons could be involved in transport of PrP^{Sc}.

Oral challenge of PrP^{Sc} in rodents strongly suggested that infection spread from the gastrointestinal tract via the splanchnic and vagus nerves to the spinal cord and brain [2, 3, 15, 22, 23]. Recently, the same result was demonstrated in the cattle after an oral challenge of PrP^{Sc} [12]. After oral ingestion of scrapie agent in the hamster, initial infection of the brain occurred via the vagus nerve rather than by spread along the spinal cord [3]. The results of the present study suggest that the transporting pathways of PrP^{Sc} in the cattle are mainly constructed by the sympathetic postganglionic neurons and partially by the sympathetic sensory neurons (Fig. 3B). The vagus nerve does not seem to contribute to the transport of PrP^{Sc} from the ileum directly. There may be differences between the hamster and cattle in the pathways along which infection spreads from the intestine to the brain. On the other hand, it is demonstrated that the vagus nerve contributes to the transport of PrP^{Sc} in the cattle [12]. The results of the present study suggest that the site of neuroinvasion to the vagus nerve may be in upper intestine.

Since HRP is taken up by nerve terminals or cell bodies, it is anterogradely and retrogradely conveyed in neurons by the axonal flow. However, there seems to be a consensus among experimental neuroanatomists that there is no significant transport of HRP from a labeled cell body to surrounding neurons. The mode of intraneuronal and interneuronal transport for PrP^{Sc} remains to be discovered [11].

ACKNOWLEDGEMENTS. This study was partly supported by a Grant-in-Aid from the BSE Control Project of the Ministry of Agriculture, Forestry and Fisheries, and by a Grant-in-Aid for Scientific Research (B) (No. 17380180) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (N. I.).

REFERENCES

1. Altschuler, S. M., Ferenci, D. A., Lynn, R. B. and Miselis, R. R. 1991. Representation of the cecum in the lateral dorsal motor nucleus of the vagus nerve and commissural subnucleus of the nucleus tractus solitarii in rat. *J. Comp. Neurol.* **304**: 261–274.
2. Baldauf, E., Beekes, M. and Diringer, H. 1997. Evidence for an alternative direct route of access for the scrapie agent to the brain bypassing the spinal cord. *J. Gen. Virol.* **78**: 1187–1197.
3. Beekes, M., McBride, P. A. and Baldauf, E. 1998. Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. *J. Gen. Virol.* **79**: 601–607.
4. Bounias, M. and Purdey, M. 2002. Transmissible spongiform encephalopathies: a family of etiologically complex diseases—a review. *Sci. Total Environ.* **297**: 1–19.
5. Brtva, R. D., Iwamoto, G. A. and Longhurst, J. C. 1989. Distribution of cell bodies for primary afferent fibers from the stomach of the cat. *Neurosci. Lett.* **105**: 287–293.
6. Bruce, M. E., Will, R. G., Ironside, J. W., McConnell, I., Drummond, D., Suttie, A., McCardle, L., Chree, A., Hope, J., Birkett, C., Cousens, S., Fraser, H. and Bostock, C. J. 1997. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. *Nature* **389**: 498–501.
7. Carp, R. I., Kascsak, R. J., Wisniewski, H. M., Merz, P. A., Rubenstein, R., Bendheim, P. and Bolton, D. 1989. The nature of the unconventional slow infection agents remains a puzzle. *Alzheimer Dis. Assoc. Disord.* **3**: 79–99.
8. Chiocchetti, R., Grandis, A., Bombardi, C., Lucchi, M. L., Dal Lago, D. T., Bortolami, R. and Furness, J. B. 2006. Extrinsic and intrinsic sources of calcitonin gene-related peptide immunoreactivity in the lamb ileum: a morphometric and neurochemical investigation. *Cell Tissue Res.* **323**: 183–196.
9. Furness, J. B. 2006. The organisation of the autonomic nervous system: Peripheral connections. *Auton. Neurosci.* **130**: 1–5.
10. Hayakawa, T., Kuwahara, S., Maeda, S., Tanaka, K. and Seki, M. 2006. Direct synaptic contacts on the myenteric ganglia of the rat stomach from the dorsal motor nucleus of the vagus. *J. Comp. Neurol.* **498**: 352–362.
11. Heikenwalder, M., Julius, C. and Aguzzi, A. 2007. Prions and peripheral nerves: A deadly rendezvous. *J. Neurosci. Res.* **85**: 2714–2725.
12. Hoffmann, C., Ziegler, U., Buschmann, A., Weber, A., Kupfer, L., Oelschlegel, A., Hammerschmidt, B. and Groschup, M. H. 2007. Prions spread via the autonomic nervous system from the gut to the central nervous system in cattle incubating bovine spongiform encephalopathy. *J. Gen. Virol.* **88**: 1048–1055.
13. Jackson, G. S. and Collinge, J. 2000. Prion disease—the propa-

gation of infectious protein topologies. *Microbes Infect.* **2**: 1445–1449.
14. Khurana, R. K. and Petras, J. M. 1991. Sensory innervation of the canine esophagus, stomach, and duodenum. *Am. J. Anat.* **192**: 293–306.
15. Kimberlin, R. H. and Walker, C. A. 1989. Pathogenesis of scrapie in mice after intragastric infection. *Virus Res.* **12**: 213–220.
16. Kirchgessner, A. L. and Gershon, M. D. 1989. Identification of vagal efferent fibers and putative target neurons in the enteric nervous system of the rat. *J. Comp. Neurol.* **285**: 38–53.
17. Kuntz, A. 1938. The structural organization of the celiac ganglia. *J. Comp. Neurol.* **69**: 1–12.
18. Kuramoto, H. and Furness, J. B. 1989. Distribution of enteric nerve cells that project from the small intestine to the coeliac ganglion in the guinea-pig. *J. Auton. Nerv. Syst.* **27**: 241–248.
19. LaVail, M. M. and LaVail, J. H. 1975. Retrograde intraaxonal transport of horseradish peroxidase in retinal ganglion cells of the chick. *Brain Res.* **85**: 273–280.
20. Luckensmeyer, G. B. and Keast, J. R. 1994. Projections from the prevertebral and major pelvic ganglia to the ileum and large intestine of the male rat. *J. Auton. Nerv. Syst.* **49**: 247–259.
21. Mabbott, N. A. and MacPherson, G. G. 2006. Prions and their lethal journey to the brain. *Nature Rev.* **4**: 201–211.
22. McBride, P. A. and Beekes, M. 1999. Pathological PrP is abundant in sympathetic and sensory ganglia of hamsters fed with scrapie. *Neurosci. Lett.* **265**: 135–138.
23. McBride, P. A., Schulz-Schaeffer, W. J., Donaldson, M., Bruce, M., Diringer, H., Kretzschmar, H. A. and Beekes, M. 2001. Early spread of scrapie from the gastrointestinal tract to the central nervous system involves autonomic fibers of the splanchnic and vagus nerves. *J. Virol.* **75**: 9320–9327.
24. Mesulam, M.-M. 1978. Tetramethyl benzidine for horseradish peroxidase neurohistochemistry: a non-carcinogenic blue reaction-product with superior sensitivity for visualizing neural afferents and efferents. *J. Hictochem. Cytochem.* **26**: 106–117.
25. Price, P. and Fisher, A. W. F. 1978. Electron microscopical study of retrograde axonal transport of horseradish peroxidase in the supraoptico-hypophyseal tract in the rat. *J. Anat.* **125**: 137–147.
26. Satomi, H., Yamamoto, T., Ise, H. and Takatama, H. 1978. Origins of the parasympathetic preganglionic fibers to the cat intestine as demonstrated by the horseradish peroxidase method. *Brain Res.* **151**: 571–578.
27. Stöckel, K., Schwab, M. and Thoenen, H. 1975. Comparison between the retrograde axonal transport of nerve growth factor and tetanus toxin in motor, sensory and adrenergic neurons. *Brain Res.* **99**: 1–16.
28. Szurszewski, J. H. and Miller, S. M. 1994. Physiology of prevertebral ganglia. pp. 795–877. *In*: Physiology of the Gastrointestinal Tract, 3rd ed. (Johnson, L. R. ed.), Raven Press, New York.
29. Taniguchi, Y. 1986. Distribution of the dorsal root ganglion cell bodies innervating the abdominal and pelvic viscera in the monkey. *Fukuoka Acta Med.* **77**: 500–525 (in Japanese).
30. Terry, L. A., Marsh, S., Ryder, S. J., Hawkins, S. A. C., Wells, G. A. H. and Spencer, Y. I. 2003. Detection of disease-specific PrP in the distal ileum of cattle exposed orally to the agent of bovine spongiform encephalopathy. *Vet. Rec.* **152**: 387–392.
31. Timmermans, J.-P., Barbiers, M., Scheuermann, D. W., Stach, W., Adriaensen, D. and De Groodt-Lasseel, M. H. A. 1993. Occurrence, distribution and neurochemical features of small intestinal neurons projecting to the cranial mesenteric ganglion in the pig. *Cell Tissue Res.* **272**: 49–58.
32. Turner, P. T. and Harris, A. B. 1974. Ultrastructure of exogenous peroxidase in cerebral cortex. *Brain Res.* **74**: 305–326.
33. Yao, Y., Tamamaki, N., Nakagawara, G. and Nojyo, Y. 1996. Distribution of vagal preganglionic neurons in the rat brain innervating thoracic and abdominal organs revealed by retrograde DiI tracing. *Acta Anat. Nippon.* **71**: 662–673.