Originally published as:

Christine Kratzel, Dominique Krüger and Michael Beekes.
Prion propagation in a nerve conduit model containing segments devoid of axons
(2007) Journal of General Virology, 88 (12), pp. 3479-3485.

DOI: 10.1099/vir.0.83187-0

Prion propagation in a nerve conduit model containing segments devoid of axons

Christine Kratzel, Dominique Krüger and Michael Beekes

Robert Koch Institute, Nordufer 20, D-13353 Berlin, Germany

Abstract

Prions, the putative causative agents of transmissible spongiform encephalopathies, are neurotropic pathogens that spread to the central nervous system via synaptically linked neural conduits upon peripheral infection. Axons and their transport processes have been suggested as mediators of nerve-associated prion dissemination. However, the exact cellular components and molecular mechanisms underlying neural spread are unknown. This study used an established hamster scrapie model to pursue a novel experimental approach using nerve conduits containing segments devoid of neurites generated by incomplete nerve regeneration following Wallerian degeneration to probe the necessity of axons for the neural propagation of prions. For this purpose, animals were subjected to unilateral sciatic neurectomy 4 weeks before footpad inoculation with scrapie agent. The results showed that the regional nerve is the prime conduit for cerebral neuroinvasion and revealed, as evidenced by the accumulation of pathological prion protein PrP^{TSE}, that prions can proceed along segments of peripheral neural projections without detectable axonal structures.

Introduction

Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of the central nervous system (CNS) in animals and humans, and include scrapie, bovine spongiform encephalopathy (BSE), chronic wasting disease (CWD), and Creutzfeldt–Jakob disease (CJD) and its variant form, vCJD. The pathognomonic feature of TSEs is the deposition in the CNS of an aberrant form of the prion protein (PrP) with a pathologically altered folding and/or aggregation structure. Disease-associated forms of the prion protein, here collectively referred to as PrP^{TSE} (Brown & Cervenakova, 2005; Beekes & McBride, 2007), can be detected in affected animals and humans by analytical methods such as Western blotting (Beekes *et al.*, 1996; Wadsworth *et al.*, 2001), immunohistochemistry (McBride *et al.*, 2001; Ironside *et al.*, 2002) and paraffin-embedded tissue (PET) blotting (Schulz-Schaeffer *et al.*, 2000), and have been established in many studies as a reliable biochemical marker for infectious TSE agents or 'prions' (McKinley *et al.*, 1983; Gabizon *et al.*, 1987; Bolton *et al.*, 1991; Jendroska *et al.*, 1991; Rubenstein *et al.*, 1991; Beekes *et al.*, 1996; Baldauf *et al.*, 1997). The prion hypothesis holds that the causative agents of TSEs are proteinaceous infectious particles (prions) that consist essentially – if not entirely – of abnormal protease-resistant prion protein (Prusiner, 1991).

During the past few years, comprehensive research on the pathogenesis of peripherally acquired TSE infections has achieved substantial progress in dissecting the pathways by which prions spread through the body. These studies imply that prions spread from peripheral sites of infection along synaptically linked neural conduits to target areas in the brain and spinal cord (Beekes *et al.*, 1998; Groschup *et al.*, 1999; McBride *et al.*, 2001; Bartz *et al.*, 2002; Beekes & McBride, 2007). Although such defined dissemination along neuroanatomical projections is suggestive of axons as a track for prions, hard data on the cellular components and molecular mechanisms that mediate the spread of TSE agents in the nervous system are scant. Several studies found that propagation of infection in various components of the nervous system takes place at a net rate of 0.7–2 mm day^{-1}, which would be consistent with transport by slow axonal flow (Kimberlin *et al.* 1983; Glatzel & Aguzzi, 2000a). PrP^{TSE} deposits have been detected in nerve fibres of parenterally infected scrapie hamsters between the myelin sheath and axon (Groschup *et al.*, 1999) and described to be present within axons in brain samples from CJD patients (Kovacs *et al.*, 2005). However, transgenic mice overexpressing four-repeat tau with a reported impairment of axonal transport displayed comparable incubation times to control mice following intraneural infection with scrapie (Künzi *et al.*, 2002). Also, the incubation time after peripheral prion infection was not found to be altered in mice with a heterozygous mutation of

dynein, a motor protein involved in the transport of axonal cargo along the cytoskeleton (Hafezparast *et al.*, 2005). In addition, pharmacological segregation of axonal neurofilaments and microtubuli by *β*,*β′*-iminodiproprionitrile did not influence the observed rate of spread of infection along peripheral nerves after administration of scrapie agent into the footpad of hamsters (Kratzel *et al.*, 2007a). Thus, apparently conflicting findings argue in favour or against axons and their transport mechanisms as key mediators of nerve-associated prion dissemination. In contrast, the axonal movement of cellular PrP has been well established (Borchelt *et al.*, 1994; Butowt *et al.*, 2006).

Here, we used sciatic neurectomy as an experimental tool to generate *in vivo* incomplete nerve conduits containing segments depleted or devoid of axons, allowing further scrutinization of the host components necessary for the propagation of prions along neural pathways. Surprisingly, examination of the model conduits produced by our approach revealed that prion propagation seems to be possible along nerve structures that do not contain detectable axonal components.

Methods

Manipulations of neural structures in animals.

The animal experiments complied with German legal regulations and were approved by the responsible authorities. For neurectomy, animals were anaesthetized with a ketamine/xylazine mixture [100 and 5 mg (kg body weight)$^{-1}$, respectively]. After shaving and skin disinfection, the right distal sciatic nerve was excavated at the femoral level by surgical dislocation of the biceps femoris muscle. The nerve was gently swabbed with 2% lidocaine. Dissection was performed using a thermo-coagulator and a nerve segment of about 3 mm was removed. The anatomical structures were repositioned and the skin was closed with a Vicryl 5.0 suture.

Mock-operated control animals were anaesthetized and skin suture was carried out at the femoral level on the right hind leg.

Animal inoculation.

Adult male and female Syrian hamsters were sedated with isoflurane and infected with scrapie by inoculation into the footpad. For infection with two different concentrations of prions, 20 µl of a 0.001% (w/v, ‘low dose’) or 2% (w/v, ‘high dose’) scrapie 263K strain hamster brain homogenate from terminally ill donor animals were injected into the sole of the right foot using a 27-gauge cannula. The animals were regularly monitored for clinical signs of scrapie (Kratzel *et al.*, 2007a). When terminally affected with scrapie, or when the experiment was terminated at 314 days post-infection (p.i.), the hamsters were sacrificed by CO_2 asphyxiation.

Experimental groups and tissue collection.

Footpad infections with low or high doses of scrapie agent were performed at 4 weeks after mock operation (n=7 for each of the low- and high-dose groups) or neurectomy (n=8 for each of the low- and high-dose groups). Non-neurectomized negative-control animals (n=4) were similarly mock infected with a 2% (w/v) normal hamster brain homogenate. One animal in the mock-operated high-dose group died before termination of the experiment for reasons unrelated to TSE disease. For PET blot detection of cerebral PrP^{TSE}, brains were removed from animals, and for immunohistological examinations of axonal cytoskeleton components (i.e. tubulin and neurofilaments), sciatic nerve samples were obtained from two neurectomized hamsters that succumbed to terminal scrapie at 155 days after infection with a high dose of scrapie agent. These nerve specimens, and the brains for PET blotting, were fixed overnight immediately after collection by immersion in 4% (v/v) formaldehyde. After incubation in PBS (pH 7.2), tissue samples were transferred to 70% ethanol, processed in a tissue processor and subsequently embedded in paraffin wax. Nerve specimens from four neurectomized animals that developed terminal scrapie after footpad infection with a high dose of scrapie agent were collected for Western blot testing for PrP^{TSE}. These specimens were frozen at −80 °C immediately after preparation. For the Western blots, sciatic nerve specimens were further dissected into proximal

nerve stumps, medial nerve tubes and distal nerve stumps. Every precaution was taken to avoid cross-contamination.

Western blotting.

Tissue extraction of PrP^{TSE} in the form of protease-resistant PrP27–30 was initiated by collagenase digestion of samples for 1 h and subsequently performed as described previously (Thomzig *et al.*, 2003). The extracts were subjected to PAGE and Western blotting using the anti-PrP monoclonal antibody (mAb) 3F4 (Kascsak *et al.*, 1987) as described in detail elsewhere (Thomzig *et al.*, 2003). Samples from uninfected hamsters spiked with 263K scrapie brain homogenate from terminally ill donors were used as positive controls for the extraction procedure, as specified in the legend to Fig. 5⇓.

Immunohistochemistry and PET blotting.

For post-mortem microscopic examination of the status of unmanipulated and dissected nerves, cross-sections of sciatic tissue samples were immunohistochemically stained using mAbs for neurofilaments (2F11; DakoCytomation) and tubulin (Abcam) diluted 1:50 and 1:200 in PBS, respectively. Fluorescent-labelled secondary antibodies (Oregon Green and Texas Red, as appropriate) were diluted 1:100.

PET blotting of brain sections was performed according to the method of Schulz-Schaeffer *et al.* (2000) with slight modifications. In brief, 6 µm thick sections were dried on nitrocellulose membranes overnight at 55 °C. After deparaffinization, sections were digested overnight with 15 µg proteinase K ml^{-1} in digestion buffer containing 10 mM Tris/HCl (pH 7.8), 30 mM NaCl and 0.1% (w/v) Brij-35 detergent. Sections were denatured in 3 M guanidine thiocyanate and blocked with 0.2% (w/v) casein before labelling with the primary mAb 3F4 diluted 1:2500. Binding of the secondary antibody diluted 1:2000 was visualized using NBT/BCIP (AppliChem).

Results

Prion dissemination to the brain and development of clinical disease

Neurectomy and mock operation were well tolerated by all animals. As expected, immediately after neurectomy, hamsters showed a complete loss of motor function and sensation distal to the tarsus. Footpad infection with scrapie agent was performed at 4 weeks after the neurectomy in order to avoid residual inflammatory processes possibly interfering with scrapie pathogenesis (Heikenwälder *et al.*, 2005; Kratzel *et al.*, 2007b) and to ensure axonal degeneration distal to the transection side.

After footpad infection of hamsters with the low dose of scrapie agent, six out of seven mock-operated animals developed clinical signs of scrapie and reached the terminal stage of disease between 219 and 308 days p.i. (Fig. 1a⇓). Spot-checked animals from this group including the non-diseased hamster (n=5) showed PrP^{TSE} accumulation in the brain as evidenced by PET blotting (Fig. 2a⇓). In contrast, none of the eight neurectomized animals developed clinical signs of scrapie after the low-dose challenge up to termination of the experiment at 314 days p.i. (Fig. 1a⇓). Only one of these animals showed the start of cerebral PrP^{TSE} deposition, whereas the other seven animals produced negative results following PET blotting for PrP^{TSE} (Fig. 2b⇓).

All animals, whether neurectomized or mock-operated controls, subjected to a high-dose scrapie infection via the footpad developed disease-specific terminal symptoms of scrapie and displayed widespread accumulation of PrP^{TSE} in cerebral PET blots (not shown). The incubation time for mock-operated control animals was 112±3 days p.i. (mean±sd) compared with 182±9 days p.i, for neurectomized animals (P<0.0001, two-tailed unpaired Student's t-test, Fig. 1b⇑).

Hamsters mock-challenged with normal brain homogenate remained free of scrapie symptoms until termination of the experiment.

Status of nerve regeneration

Post-mortem macroscopic examination of sciatic nerves from all neurectomized animals revealed the process of nerve regeneration. Nerve-like tubes between proximal and distal nerve stumps could be observed and proximal stumps often showed a neuroma-like formation (Fig. 3⇓).

Cross-sections of nerve specimens from two animals inoculated with a high dose of scrapie agent were examined immunohistochemically in more detail with particular attention on the axonal regeneration status after having succumbed to terminal scrapie at 155 days p.i. (Fig. 4a–h⇓). Control nerve segments from the unmanipulated contralateral side of these animals showed regular immunofluorescent staining of axonal key components such as tubulin and neurofilaments (Fig. 4a and e⇓). Staining of nerve segments from the neurectomized ipsilateral side revealed strong but disordered signals for tubulin and neurofilament in the proximal nerve stumps (Fig. 4b and f⇓), weak signals in the medial nerve tubes (Fig. 4c and g⇓) and no signals for the tested axonal components in the distal stump (Fig. 4d and h⇓).

Western blot detection of PrP^{TSE} in regenerated nerve conduits

Nerve specimens from four neurectomized animals that developed terminal scrapie after footpad infection with a high dose of scrapie agent were tested by Western blotting for PrP^{TSE}. The biochemical marker of prion infectivity could be detected in the proximal stumps, in the medial portions and in the distal stumps of the regenerating sciatic nerves (Fig. 5⇓).

Discussion

A variety of studies have shown that peripheral prion infections proceed to the CNS along neural conduits (Fraser, 1982; Beekes *et al.*, 1998; Groschup *et al.*, 1999; McBride *et al.*, 2001; Bartz *et al.*, 2002; Beekes & McBride, 2007). This, and other findings, has led to the suggestion that axons are essentially involved in the neural spread of prions and, more specifically, that axonal transport mechanisms may mediate this process. However, several experimental approaches have produced apparently contradictory results on the role of axons (Künzi *et al.*, 2002; Hafezparast *et al.*, 2005; Kovacs *et al.*, 2005; Kratzel *et al.*, 2007a), and other modes of spread of prions along neural projections are conceivable. Among these might be cell-free or cell-associated dissemination in perineural lymphatics as well as in neural interspaces (Mims & White, 1984) or sequential infection of Schwann cells (Follet *et al.*, 2002). Alternatively, exosomal vehicles for transmission of the infectious prion protein (Février *et al.*, 2005) or internalization of secreted forms of PrP by neighbouring cells (Butowt *et al.*, 2006) have been discussed, and Glatzel & Aguzzi (2000b) suggested a domino-like conversion of cellular PrP into PrP^{TSE}.

In order to establish whether prion propagation in peripheral neural tissue is feasible without an intact axonal anatomy or any detectable axonal structures at all, we used a functional sciatic nerve model, which constituted a neural conduit containing segments depleted or devoid of axons. Neurectomy of regional nerves was carried out previously for studies on the pathogenesis of neurotropic infectious diseases (Bassant *et al.*, 1986; Carbone *et al.*, 1987). However, to the best of our knowledge, nerve segments devoid of axons purposefully induced by experimental surgery have not been used to date to probe the necessity of neurites in the spread of prions through the body.

Sciatic neurectomy as performed in our hamster model is known to induce Wallerian degeneration in the distal nerve stumps (Waller, 1850; Burnett & Zager, 2004). After a latency period of a few days, physical fragmentation of both axons and myelin occurs in the disconnected distal stump. Axon and myelin debris are phagocytosed by Schwann cells and macrophages. Finally, only nerve fibre remnants consisting of Schwann cells within an endoneurial sheet remain in the distal stump. This degenerative process is usually completed within a few weeks. Nerve regeneration may be initiated by Schwann cell proliferation starting from the proximal stump, leading to continuous columns of cells known as the bands of Büngner (Büngner, 1891). These bands provide a guide for axons that can sprout from the proximal stump into surrounding tissue and eventually grow into the distal stump (Ide, 1996; Burnett & Zager, 2004). However, after transection, such regenerating axons are no longer guided by their original sheaths and are thus likely to fail functionally correct reinnervation.

In our study, the effect of sciatic neurectomy observed upon footpad infection of hamsters with a low dose of scrapie agent was pronounced (Fig. 1a⇑). Six out of seven mock-operated hamsters developed terminal scrapie between 219 and 308 days p.i. In contrast, all neurectomized animals remained free of symptoms until 314 days p.i. when the experiment for this group was terminated, and only one of these animals showed weak deposition of PrP^{TSE} in the brain. These effects of neurectomy functionally demonstrated that the nerve innervating the site of infection provided the prime conduit for prions to the CNS after footpad inoculation. For hamsters subjected to neurectomy of the sciatic nerve before high-dose scrapie footpad infection, we observed a prolongation of the survival time of about 60% compared with mock-operated control animals (Fig. 1b⇑). The difference between the observed incubation times without and with neurectomy (112 vs 182 days) approximately corresponded to that observed in incubation time-interval assays after intracerebral inoculation of 1.5×10^2 LD_{50} and a 100-fold lower dose, respectively (not shown).

However, despite substantially prolonged survival after sciatic neurectomy, all animals of the high-dose group succumbed to terminal disease. How did the infection spread to the CNS in these animals? Centripetal spreading pathways via non-sciatic neural projections, lymph or blood may provide an explanation. However, excision of the draining lymph node 4 weeks before footpad inoculation of non-neurectomized hamsters with 2% 263K scrapie brain homogenate did not alter survival time (Kratzel *et al.*, 2007b). Alternatively, delayed invasion of the brain could be accounted for by prion propagation from the distal nerve stump after partial sciatic regeneration. This prompted us to examine the disrupted parts of the sciatic nerve in more detail. An inspection of the operation site revealed that all neurectomized hamsters developed macroscopically visible tube-like structures connecting the proximal and distal stumps of the transected nerves (Fig. 3⇑), although functional recovery was not observed in any of the neurectomized animals. Immunohistochemical examination with antibodies specific to key components of the axonal cytoskeleton such as tubulin and neurofilaments indicated sprouting axons in the proximal neuroma, growing distally to the level of the former nerve transection (Fig. 4b–d and f–h⇑). No evidence of axons could be detected in the examined distal stumps of terminally ill hamsters from the high-dose group sacrificed at 155 days p.i. (Fig. 4d and h⇑). In contrast, when we tested regenerated nerve samples from scrapie hamsters between 150 and 212 days p.i. for PrP^{TSE} by highly sensitive Western blotting (Fig. 5⇑), PrP^{TSE} was consistently found in the proximal and medial portion of the injured nerve, and also in the distal nerve stumps.

The footpad inoculations of scrapie agents were performed 4 weeks after the neurectomy when Wallerian degeneration in the distal nerve stump was fairly advanced, and previous studies have shown that, even in intact sciatic nerves, PrP^{TSE} becomes detectable only at 60 days following footpad infection (Kratzel *et al.*, 2007a). Thus, the accumulation of PrP^{TSE} in the distal nerve stumps cannot represent a remnant from prion propagation in the distal nerve stumps occurring prior to Wallerian degeneration, but must have been sustained by neural components other than axons.

Schwann cells have been shown to express cellular PrP in murine nerves and displayed PrP^{TSE} formation after exposure to scrapie agent in culture (Follet *et al.*, 2002). Furthermore, Herzog *et al.* (2004) reported the detection of PrP^{TSE} in Schwann cells of the sciatic nerve from primates peripherally infected with BSE agent. In the peripheral nervous system (PNS), these glial cells ensheath axonal projections as well as synaptic junctions (Fields & Stevens-Graham, 2002). Against this background, the prion propagation we observed in axon-free neural repair tissue of distal nerve stumps can plausibly be explained by an involvement of Schwann cells. However, other modes of non-axonal spread cannot be ruled out as yet and also need to be addressed in future studies. Additionally, it must be emphasized that our findings do not necessarily argue against a key role for axonal conduits for the spread of prions along neural projections. Rather, they hint at an alternative or additional mode of propagation that may operate in parts of the PNS and possibly involves Schwann cells. Evidently, the role of glial cells and other non-neuronal components in the spread of TSE agents along PNS projections requires further elucidation – for example, by studies using transgenic mice, by pharmacological approaches or by using the model system of neural conduits devoid of axons described in this report.

Acknowledgments

We thank Kristin Kampf, Marion Joncic and Patricia Reckwald for their excellent technical assistance and Kazimirz Madela for the laser scanning micrographs. The work was supported by the European

Union (contract no. QLG3-CT-2002-81030). C.K. is particularly grateful to the Robert Koch Institute (Berlin) for continuous support and M.B. would like to thank the EU-funded Network of Excellence ‘NeuroPrion’.

References

Baldauf, E., Beekes, M. & 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.
Bartz, J. C., Kincaid, A. E. & Bessen, R. A. (2002). Retrograde transport of transmissible mink encephalopathy within descending motor tracts. J Virol 76, 5759–5768.
Bassant, M. H., Baron, H., Gumpel, M., Cathala, F. & Court, L. (1986). Spread of scrapie agent to the central nervous system: study of a rat model. Brain Res 383, 397–401.
Beekes, M. & McBride, P. A. (2007). The spread of prions through the body in naturally acquired transmissible spongiform encephalopathies. FEBS J 274, 588–605.
Beekes, M., Baldauf, E. & Diringer, H. (1996). Sequential appearance and accumulation of pathognomonic markers in the central nervous system of hamsters orally infected with scrapie. J Gen Virol 77, 1925–1934.
Beekes, M., McBride, P. A. & Baldauf, E. (1998). Cerebral targeting indicates vagal spread of infection in hamsters fed with scrapie. J Gen Virol 79, 601–607.
Bolton, D. C., Rudelli, R. D., Currie, J. R. & Bendheim, P. E. (1991). Copurification of Sp33–37 and scrapie agent from hamster brain prior to detectable histopathology and clinical disease. J Gen Virol 72, 2905–2913.
Borchelt, D. R., Koliatsos, V. E., Guarnieri, M., Pardo, C. A., Sisodia, S. S. & Price, D. L. (1994). Rapid anterograde axonal transport of the cellular prion glycoprotein in the peripheral and central nervous system. J Biol Chem 269, 14711–14714.
Brown, P. & Cervenakova, L. (2005). A prion lexicon (out of control). Lancet 365, 122.
Büngner, O. V. (1891). U¨ ber die Degenerations- und Regenerationsvorgänge am Nerven nach Verletzungen. Beitr Pathol Anat 10, 321–387.
Burnett, M. G. & Zager, E. L. (2004). Pathophysiology of peripheral nerve injury: a brief review. Neurosurg Focus 16, E1.
Butowt, R., Abdelraheim, S., Brown, D. R. & von Bartheld, C. S. (2006). Anterograde axonal transport of the exogenous cellular isoform of prion protein in the chick visual system. Mol Cell Neurosci 31, 97–108.
Carbone, K. M., Duchala, C. S., Griffin, J. W., Kincaid, A. L. & Narayan, O. (1987). Pathogenesis of Borna disease in rats: evidence that intra-axonal spread is the major route for virus dissemination and the determinant for disease incubation. J Virol 61, 3431–3440.
Février, B., Vilette, D., Laude, H. & Rapaso, G. (2005). Exosomes: a bubble ride for prions? Traffic 6, 10–17.
Fields, R. D. & Stevens-Graham, B. (2002). New insights into neuron–glia communication. Science 298, 556–562.
Follet, J., Lemaire-Vieille, C., Blanquet-Grossard, F., Podevin-Dimster, V., Lehmann, S., Chauvin, J. P., Decavel, J. P., Varea, R.,
Grassi, J. & other authors (2002). PrP expression and replication by Schwann cells: implications in prion spreading. J Virol 76, 2434–2439.
Fraser, H. (1982). Neuronal spread of scrapie agent and targeting of lesions within the retino-tectal pathway. Nature 295, 149–150.
Gabizon, R., McKinley, M. P. & Prusiner, S. B. (1987). Purified prion proteins and scrapie infectivity copartition into liposomes. Proc Natl Acad Sci U S A 84, 4017–4021.
Glatzel, M. & Aguzzi, A. (2000a). PrPC expression in the peripheral nervous system is a determinant of prion neuroinvasion. J Gen Virol 81, 2813–2821.
Glatzel, M. & Aguzzi, A. (2000b). Peripheral pathogenesis of prion diseases. Microbes Infect 2, 613–619.
Groschup, M. H., Beekes, M., McBride, P. A., Hardt, M., Hainfellner, J. A. & Budka, H. (1999). Deposition of disease-associated prion protein involves the peripheral nervous system in experimental scrapie. Acta Neuropathol (Berl) 98, 453–457.
Hafezparast, M., Brandner, S., Linehan, J., Martin, J. E., Collinge, J. & Fisher, E. M. (2005). Prion disease incubation time is not affected in mice heterozygous for a dynein mutation. Biochem Biophys Res Commun 326, 18–22.
Heikenwälder, M., Zeller, N., Seeger, H., Prinz, M., Klöhn, P.-C., Schwarz, P., Ruddle, N. H., Weissmann, C. & Aguzzi, A. (2005).
Chronic lymphocytic inflammation specifies the organ tropism of prions. Science 307, 1107–1110.
Herzog, C., Sales, N., Etchegaray, N., Charbonnier, A., Freire, S., Dormono, D., Deslys, J. P. & Lasmezas, C. I. (2004). Tissue distribution of bovine spongiform encephalopathy agent in primates after intravenous or oral infection. Lancet 363, 422–428.
Ide, C. (1996). Peripheral nerve regeneration. Neurosci Res 25, 101–121.

Ironside, J. W., McCardle, L., Horsburgh, A., Lim, Z. & Head, M. W. (2002). Pathological diagnosis of variant Creutzfeldt–Jakob disease. APMIS 110, 79–87.
Jendroska, K., Heinzel, F. P., Torchia, M., Stowring, L., Kretzschmar, H. A., Kon, A., Stern, A., Prusiner, S. B. & DeArmond, S. J. (1991).
Proteinase-resistant prion protein accumulation in Syrian hamster brain correlates with regional pathology and scrapie infectivity. Neurology 41, 1482–1490.
Kascsak, R. J., Rubenstein, R., Merz, P. A., Tonna-DeMasi, M., Fersko, R., Carp, R. I., Wisniewski, H. M. & Diringer, H. (1987). Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J Virol 61, 3688–3693.
Kimberlin, R. H., Hall, S. M. & Walker, C. A. (1983). Pathogenesis of mouse scrapie. Evidence for direct neural spread of infection to the CNS after injection of sciatic nerve. J Neurol Sci 61, 315–325.
Kovacs, G. G., Preusser, M., Strohschneider, M. & Budka, H. (2005). Subcellular localization of disease-associated prion protein in the human brain. Am J Pathol 166, 287–294.
Kratzel, C., Mai, J., Madela, K., Beekes, M. & Krüger, D. (2007a).
Propagation of scrapie in peripheral nerves after footpad infection in normal and neurotoxin exposed hamsters. Vet Res 38, 127–139.
Kratzel, C. & Krüger, D. & Beekes, M. (2007b). Relevance of the regional lymph node in scrapie pathogenesis after peripheral infection of hamsters. BMC Vet Res 3, 22 [Epub ahead of print].
Künzi, V., Glatzel, M., Nakano, M. Y., Greber, U. F., Van Leuven, F. & Aguzzi, A. (2002). Unhampered prion neuroinvasion despite impaired fast axonal transport in transgenic mice overexpressing four-repeat tau. J Neurosci 22, 7471–7477.
McBride, P. A., Schulz-Schaeffer, W. J., Donaldson, M., Bruce, M., Diringer, H., Kretzschmar, H. A. & 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.
McKinley, M. P., Bolton, D. C. & Prusiner, S. B. (1983). A proteaseresistant protein is a structural component of the scrapie prion. Cell 35, 57–62.
Mims, C. A. & White, D. O. (1984). Viral Pathogenesis and Immunology. Oxford, UK: Blackwell Scientific. Prusiner, S. B. (1991). Molecular biology of prion diseases. Science 252, 1515–1522.
Rubenstein, R., Merz, P. A., Kascsak, R. J., Scalici, C. L., Papini, M. C., Carp, R. I. & Kimberlin, R. H. (1991). Scrapie-infected spleens: analysis of infectivity, scrapie-associated fibrils, and protease-resistant proteins. J Infect Dis 164, 29–35.
Schulz-Schaeffer, W. J., Tschoke, S., Kranefuss, N., Drose, W., Hause-Reitner, D., Giese, A., Groschup, M. H. & Kretzschmar, H. A. (2000).
The paraffin-embedded tissue blot detects PrPSc early in the incubation time in prion diseases. Am J Pathol 156, 51–56.
Thomzig, A., Kratzel, C., Lenz, G., Kru¨ ger, D. & Beekes, M. (2003). Widespread PrPSc accumulation in muscles of hamsters orally infected with scrapie. EMBO Rep 4, 530–533.
Wadsworth, J. D., Joiner, S., Hill, A. F., Campbell, T. A., Desbruslais, M., Luthert, P. J. & Collinge, J. (2001). Tissue distribution of protease resistant prion protein in variant Creutzfeldt–Jakob disease using a highly sensitive immunoblotting assay. Lancet 358, 171–180.
Waller, A. (1850). Experiments on the section of the glossopharyngeal and hypoglossal nerves of the frog, and observations of the alterations produced thereby in the structure of their primitive fibers. Philos Trans R Soc Lond 140, 423–429.

Tables and Figures

Figure 1. Effect of sciatic neurectomy on the survival of hamsters infected via the footpad with a low dose (a; 0.001%) or high dose (b; 2%) of 263K scrapie brain homogenate compared with mock-operated controls. In (b), squares and triangles represent individuals and bars represent means.

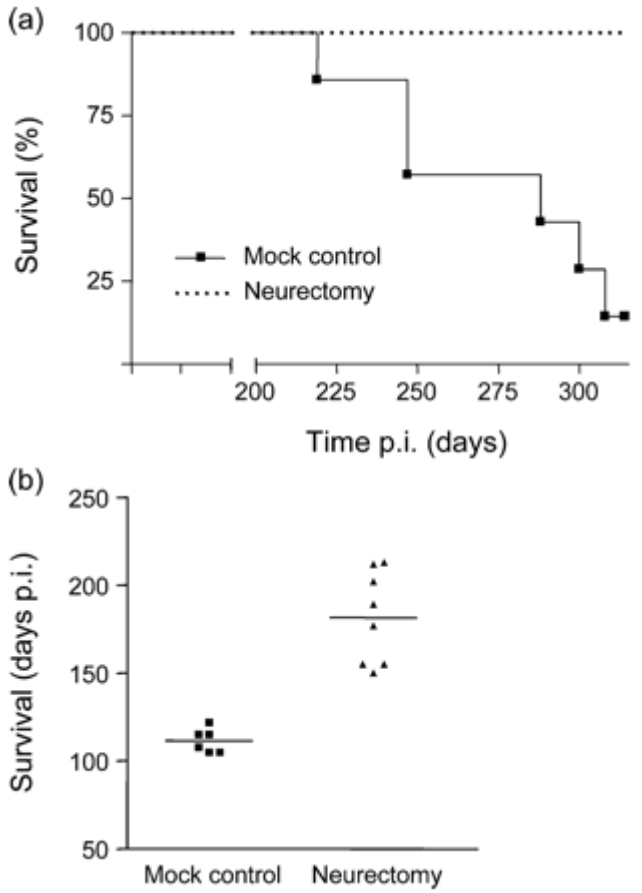

Figure 2. Representative results of PET blot testing for PrP^{TSE} in coronal brain sections from hamsters infected via the footpad with a low dose (0.001%) of 263K scrapie brain homogenate. (a) Specimen from a mock-operated hamster that succumbed to terminal disease at 299 days p.i., showing substantial immunolabelling for PrP^{TSE}. (b) Specimen from a neurectomized animal showing no clinical signs of scrapie or detectable cerebral deposition of PrP^{TSE} at 314 days p.i.

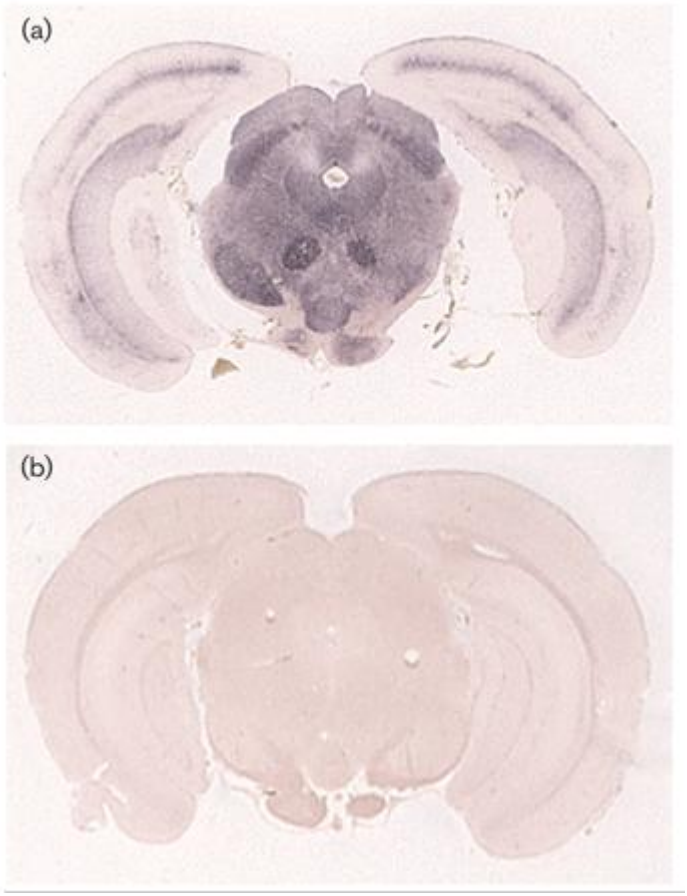

Figure 3. Representative appearance of sciatic nerve specimens excised at the site of neurectomy (arrow, right specimen) and the corresponding contralateral region (left specimen) from hamsters that developed terminal scrapie upon footpad infection with a high dose (2%) of 263K scrapie brain homogenate. The asterisk indicates the proximal, neuroma-like stump and the diamond indicates the distal nerve stump. Bar, ~1 cm.

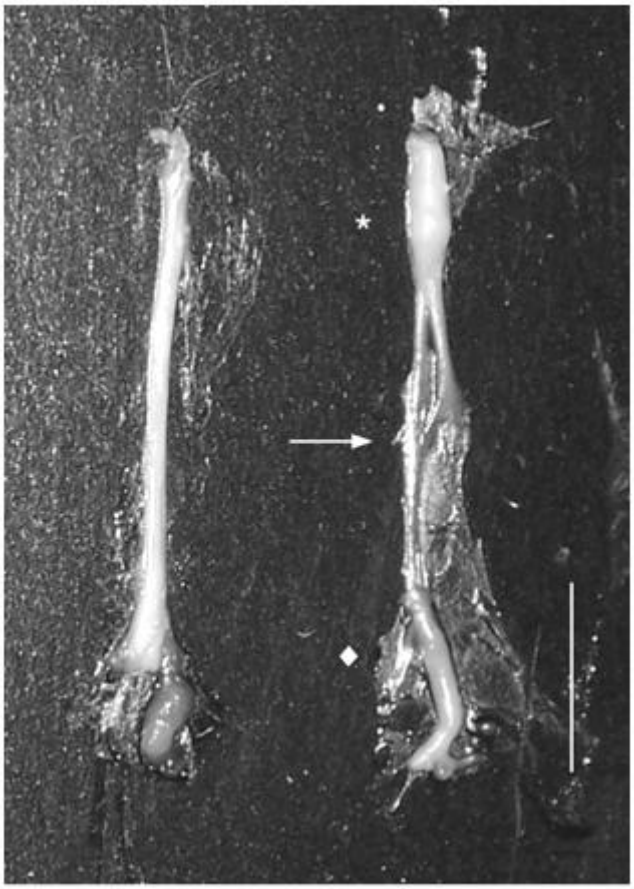

Figure 4. Photomicrographs of sciatic cross-sections representing the region of neurectomy and an unmanipulated contralateral nerve segment of a hamster that succumbed to terminal scrapie at 155 days p.i. following footpad infection with a high dose (2%) of 263K scrapie brain homogenate. Immunofluorescent labelling of tubulin (red, a–d) and neurofilaments (green, e–h) in sciatic nerve segments from the unmanipulated contralateral side (a, e) and the manipulated ipsilateral side (b–d, f–h). Proximal samples (b and f) show strong disordered signals for tubulin and neurofilaments. Weak signals for tubulin and neurofilaments were found in the nerve-like tubes between the proximal and distal nerve stumps (c and g). No signals for the tested axon components were detected in the distal stump (d and h). Images were detected by confocal laser scanning microscopy; magnification, ×400.

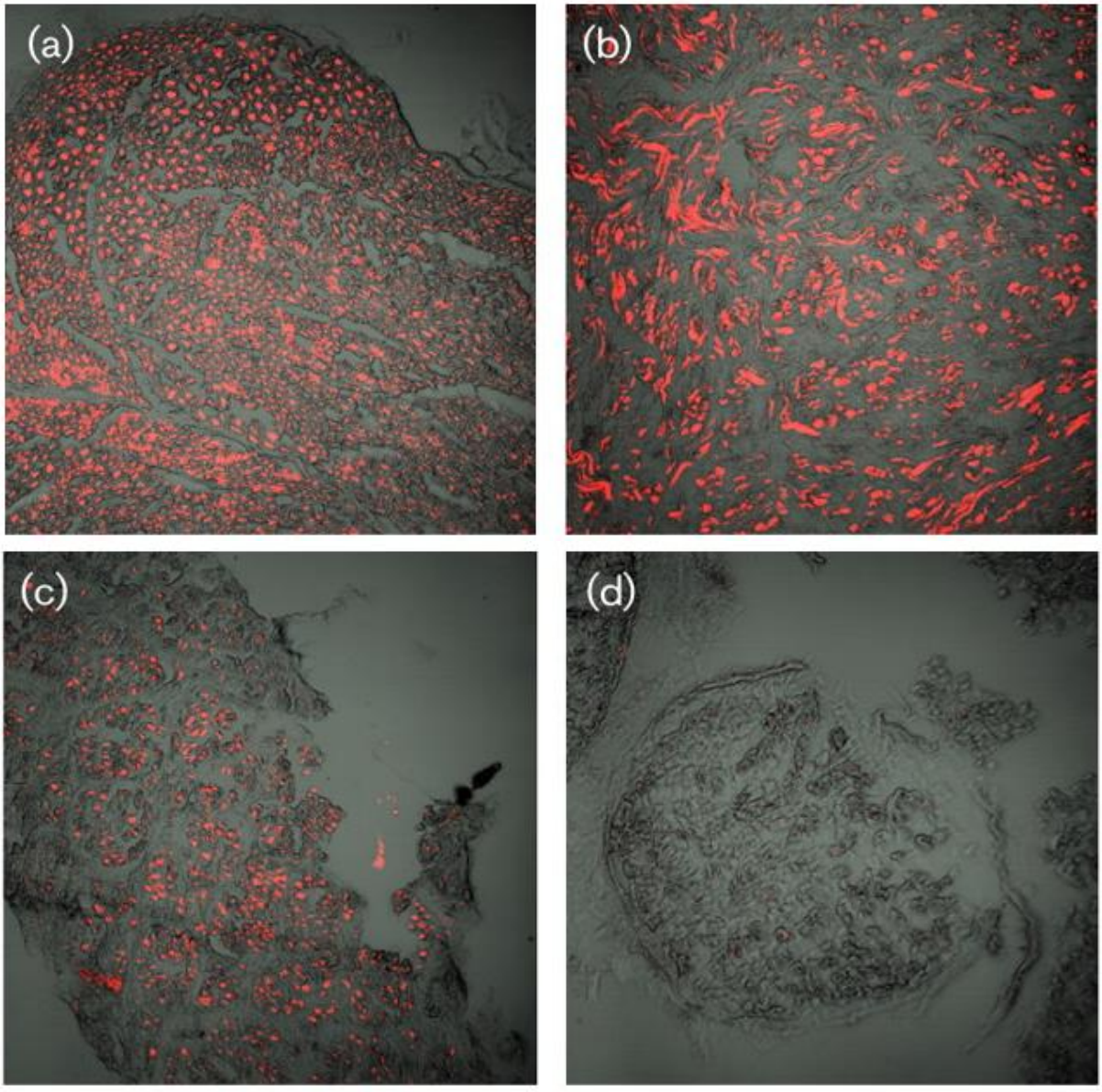

Figure 4 *(continued)*

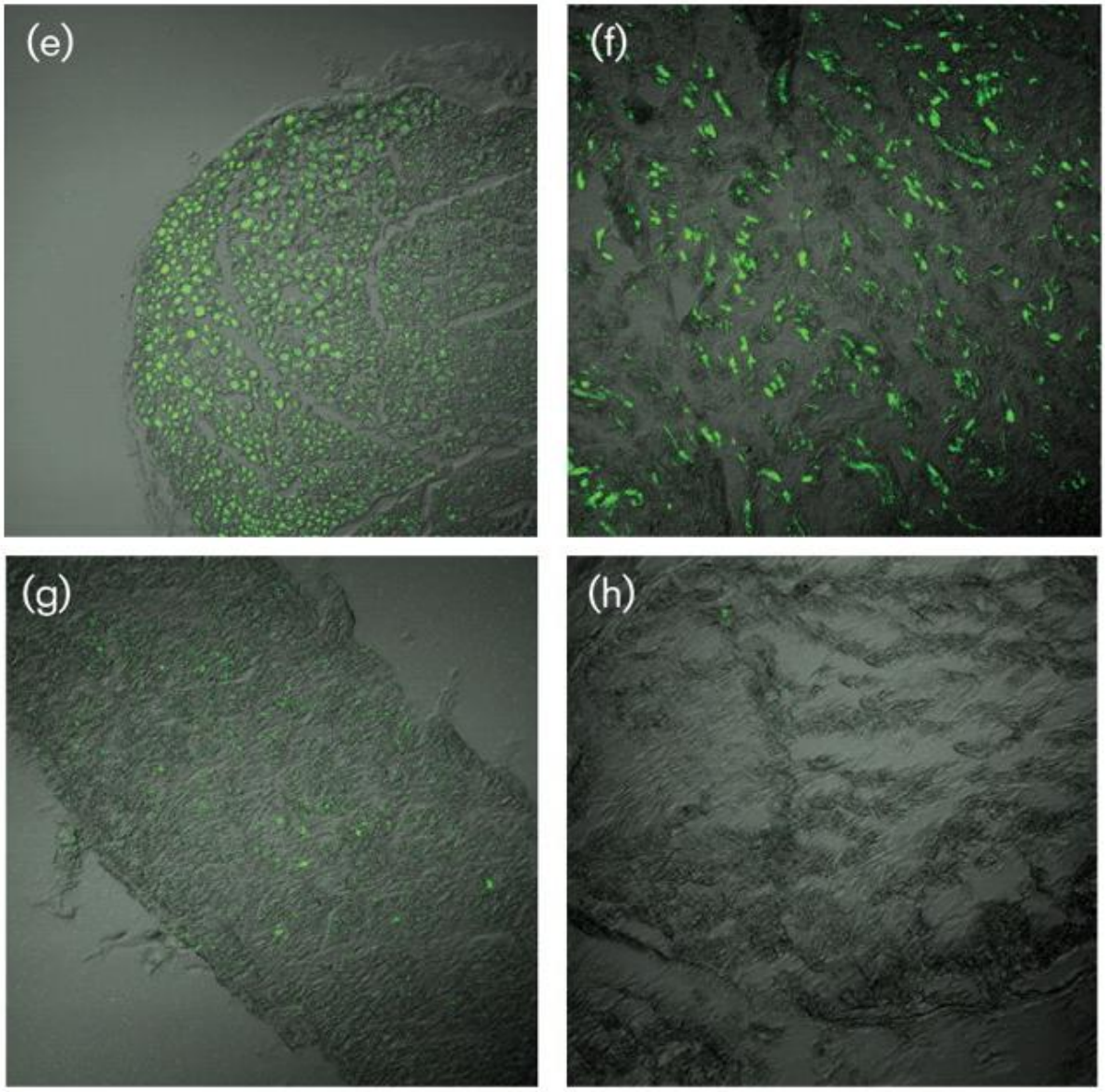

Figure 5. Representative Western blots for the detection of PrP27–30, the protease-resistant core of PrP^{TSE}, in ipsilateral sciatic nerve specimens from three hamsters that were infected in the footpad with a high dose (2%) of 263K scrapie brain homogenate after neurectomy. Due to the small and varying sample sizes of proximal and distal nerve stumps (range 1.4–3.6 mg) and of medial nerve tubes (range 0.6–0.9 mg), a precise standardization of sample loading was not possible. Thus, the Western blot signals provide a qualitative indicator for the presence of PrP^{TSE} but cannot be directly compared quantitatively. (a) Molecular mass marker (27 kDa); (b) blot control sample containing 10^{-6} g 263K scrapie brain tissue; (c, f and i) proximal nerve stumps; (d, g and j) medial nerve tubes, located between the proximal and distal nerve stumps; (e, h and k) distal nerve stumps; (l) processing control, spiked with 10^{-5} g 263K scrapie brain tissue prior to extraction of PrP^{TSE}. Sciatic samples from animals inoculated into the footpad with normal hamster brain homogenate did not produce any Western blot signals (not shown).