letters to nature

sex for all faces by the seven subjects was near 100% (mean response over all subjects and experiments: 96.6%, range: 81.3–100%).

Image acquisition and analysis. Echoplanar MR brain images were acquired using a 1.5 Tesla GE Signa system (General Electric) retrofitted with advanced NMR hardware (ANMR) using a standard head coil. 100 T2*-weighted images depicting BOLD contrast[25] were acquired over 5 min (for each experiment) at each of 14 near-axial non-contiguous 5-mm-thick planes parallel to the intercommissural (AC-PC) line, providing whole-brain coverage: TE, 40 ms; TR, 3 s; in-plane resolution, 3 mm; interslice gap, 0.5 mm. An inversion recovery EPI dataset was also acquired at 43 near-axial 3-mm-thick planes parallel to the AC-PC line: TE, 80 ms; TI, 180 ms; TR, 16 s; in-plane resolution, 3 mm; number of signal averages, 8. The periodic change in T2*-weighted signal intensity at the (fundamental) experimentally determined frequency of alternation between A and B conditions was analysed by pseudogeneralized least-squares (PGLS) fit of a sinusoidal regression model to the movement-corrected[26] time series at each voxel, yielding parametric maps of the squared amplitude of the response at the stimulus frequency divided by its standard error—the fundamental power quotient, FPQ (ref. 27). Each observed time series was randomly permuted ten times, and FPQ estimated as above in each randomized time series, to generate 10 randomized parametric maps of FPQ for each subject in each anatomical plane. To construct generic brain activation maps, showing brain regions activated over a group of subjects, observed and randomized parametric maps of FPQ estimated in each individual were first transformed into the stereotactic space of Talairach and Tournoux and smoothed by a gaussian filter with full width at half maximum of 11 mm (ref. 28). The median observed value of FPQ was then computed at each voxel in standard space and its statistical significance tested by reference to the null distribution of median FPQ computed from the identically smoothed and spatially transformed randomized maps. For a one-tailed test of size p, the critical value was the $100*(1 − p)$th percentile of the randomization distribution[29]. To identify voxels that demonstrated significant difference in standardized power of response to faces that expressed disgust with different intensities, the observed difference in median FPQ between these two experimental conditions was computed at each voxel. Subjects were then randomly reassigned to one of two equal-sized groups and the difference in median FPQ between randomized groups was computed at each voxel[30]. This process was repeated 64 times and the results were pooled over voxels to generate a null distribution for difference in median FPQ. For a two-tailed test of size p, the critical values were the $100*(1 − p/2)$th and $100*(p/2)$th percentiles of the randomization distribution.

1. Ekman, P. An argument for basic emotions. *Cog. Emot.* **6**, 169–200 (1992).
2. Adolphs, R. *et al.* Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* **372**, 669–672 (1994).
3. Adolphs, R. *et al.* Fear and the human amygdala. *J. Neurosci.* **16**, 7678–7687 (1995).
4. Calder, A. J. *et al.* Facial emotion recognition after bilateral amygdala damage: differentially severe impairment of fear. *Cogn. Neuropsychol.* **13**, 699–740 (1996).
5. Breiter, H. C. *et al.* Response and habituation of the human amygdala during visual processing of facial expression. *Neuron* **17**, 875–887 (1996).
6. Morris, J. *et al.* A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* **383**, 812–815 (1996).
7. Darwin, C. *The Expression of the Emotions in Man and Animals* (University of Chicago Press, Chicago, 1965).
8. Rozin, P. & Fallon, A. E. A perspective on disgust. *Psychol. Rev.* **94**, 23–41 (1987).
9. Rozin, P., Lowery, L. & Ebert, R. J. Varieties of disgust faces and the structure of disgust. *Pers. Soc. Psychol.* **66**, 870–881 (1994).
10. Vrana, S. R. The psychophysiology of disgust: differentiating negative emotional context with facial EMG. *Psychophysiology* **30**, 279–286 (1993).
11. Alexander, G. E. *et al.* Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* **9**, 357–381 (1986).
12. Sprengelmeyer, R. *et al.* Perception of faces and emotions: loss of disgust in Huntington's disease. *Brain* **119**, 1647–1665 (1996).
13. Gray, J. M. *et al.* Impaired recognition of disgust in Huntington's disease gene carriers. *Brain* (in the press).
14. Ekman, P. & Friesen, W. V. *Pictures of Facial Affect* (Consulting Psychologists, Palo Alto, 1976).
15. Perrett, D. I., May, K. A. & Yoshikawa, S. Facial shape and judgements of female attractiveness. *Nature* **368**, 239–242 (1994).
16. Rolls, E. T. in *Handbook of Clinical Olfaction and Gustation* (ed. Doty, R. L.) (Dekker, New York, 1994).
17. Yaxley, S. *et al.* The responsiveness of neurons in the insular gustatory cortex of the macaque monkey is independent of hunger. *Physiol. Behav.* **42**, 223–229 (1988).
18. Kinomura, S. *et al.* Functional anatomy of taste perception in the human brain studied with positron emission tomography. *Brain Res.* **659**, 263–266 (1994).
19. Casey, K. L. *et al.* Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. *J. Neurophysiol.* **71**, 802–807 (1994).
20. Barbas, H. Organization of cortical afferent input to orbitofrontal areas in the Rhesus monkey. *Neuroscience* **56**, 841–864 (1993)
21. Kosslyn, S. M. *et al.* Neural effects of visualizing and perceiving aversive stimuli: a PET investigation. *NeuroReport* **7**, 1569–1576 (1996).
22. Gentilucci, M. *et al.* Functional organization of inferior area 6 in the macaque monkey: I. Somatotopy and the control of proximal movements. *Exp. Brain Res.* **71**, 475–490 (1988).
23. Rolls, E. T. *et al.* Orbitofrontal cortex neurons: Role in olfactory and visual association learning. *J. Neurophysiol.* **75**, 1970–1981 (1996).
24. Calder, A. J., Young, A. W., Rowland, D. & Perrett, D. I. Computer-enhanced emotion in facial expressions. *Proc. R. Soc. B.* (in the press).
25. Ogawa, S., Lee, T. M., Kay, A. R. & Tank, D. W. Brain magnetic resonance imaging with contrast dependent blood oxygenation. *Proc. Natl Acad. Sci. USA* **87**, 8868–8872 (1990).
26. Friston, K. J. *et al.* Movement-related effects in fMRI time series. *Mag. Res. Med.* **35**, 346–355 (1996).
27. Bullmore, E. T. *et al.* Statistical methods of estimation and inference for functional MR image analysis. *Mag. Res. Med.* **35**, 261–277 (1996).
28. Talairach, J. & Tournoux, P. *Co-planar Stereotactic Atlas of the Human Brain* (Thieme, Stuttgart, 1988).
29. Brammer, M. J. *et al.* Generic brain activation mapping in fMRI: a nonparametric approach. *Mag. Res. Imag.* (in the press).
30. Edgington, E. S. *Randomisation Tests* (Dekker, New York, 1980).

Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent

M. E. Bruce*, R. G. Will†, J. W. Ironside†, I. McConnell*, D. Drummond*, A. Suttie*, L. McCardle†, A. Chree*, J. Hope‡, C. Birkett‡, S. Cousens§, H. Fraser* & C. J. Bostock‡

* *Institute for Animal Health, BBSRC/MRC Neuropathogenesis Unit, West Mains Road, Edinburgh EH9 3JF, UK*

† *National CJD Surveillance Unit, Western General Hospital, Edinburgh EH4 2XU, UK*

‡ *Institute for Animal Health, Compton, Newbury, Berkshire RG20 7NN, UK*

§ *Department of Epidemiology and Population Science, London School of Hygiene and Tropical Medicine, London WC1E 7HT, UK*

There are many strains of the agents that cause transmissible spongiform encephalopathies (TSEs) or 'prion' diseases. These strains are distinguishable by their disease characteristics in experimentally infected animals, in particular the incubation periods and neuropathology they produce in panels of inbred mouse strains[1–4]. We have shown that the strain of agent from cattle affected by bovine spongiform encephalopathy (BSE) produces a characteristic pattern of disease in mice that is retained after experimental passage through a variety of intermediate species[5–7]. This BSE 'signature' has also been identified in transmissions to mice of TSEs of domestic cats and two exotic species of ruminant[6,8], providing the first direct evidence for the accidental spread of a TSE between species. Twenty cases of a clinically and pathologically atypical form of Creutzfeldt–Jakob disease (CJD), referred to as 'new variant' CJD (vCJD)[9], have been recognized in unusually young people in the United Kingdom, and a further case has been reported in France[10]. This has raised serious concerns that BSE may have spread to humans, putatively by dietary exposure. Here we report the interim results of transmissions of sporadic CJD and vCJD to mice. Our data provide strong evidence that the same agent strain is involved in both BSE and vCJD.

Transmissions to mice were set up from six typical sporadic cases of CJD (spCJD) and three cases of vCJD. All were homozygous for methionine at codon 129 of the 'prion protein' (PrP) gene, and none carried PrP gene mutations associated with familial disease. The spCJD cases included two dairy farmers (aged 61 and 64 years) who had had BSE in their herds and had therefore been potentially exposed to BSE-infected cattle or contaminated animal feed[11]; two 'contemporary' cases (aged 55 and 57 years) with no known occupational exposure to BSE; and two 'historical' cases (aged 57 and 82 years) who had died in 1981 and 1983, before the onset of the

BSE outbreak. All of these spCJD cases were characterized by widespread spongiform vacuolation in the brain with few or no amyloid plaques. The vCJD cases (aged 29, 30 and 31 years) had clinical and neuropathological characteristics that were atypical for CJD[9]. The main distinguishing neuropathological features in these and other vCJD cases are an extensive deposition of PrP amyloid in the brain as large 'florid' plaques and a prominent involvement of the cerebellum.

Panels of three inbred mouse strains (RIII, C57BL and VM) and one cross (C57BL × VM) were challenged with CJD brain homogenates. Previous transmissions, using the same protocol, of BSE from eight unrelated cattle (Fig. 1b) and TSEs from three domestic cats (Fig. 1c), a greater kudu and a nyala (two exotic ruminants) have given a remarkably uniform pattern of incubation periods in these mice[5,6,8]. The shortest incubation periods were seen in RIII mice, with means ranging from 302 to 335 days for transmissions from frozen brain samples. These isolates also produced strikingly similar patterns of vacuolar degeneration in the brains of infected mice, as represented by the 'lesion profile'[5,6] (Fig. 2b, c). The lesion profile is a well-established semiquantitative method of measuring the targeting of vacuolation to different brain regions, and reliably discriminates between TSE strains in mice[2]. In addition, the disease characteristics in mice injected with brain from two sheep, a goat and a pig that had been experimentally infected with BSE were very similar to those seen in direct BSE transmissions from cattle[6,7].

The BSE 'signature', based on both incubation periods and pathology, has only ever been seen in transmissions from animals suspected or known to have been infected with BSE. It has never been seen throughout an extensive series of transmissions, set up in Edinburgh between 1963 and 1994, of other naturally occurring TSEs (35 sheep and two goats with scrapie, two mink with transmissible mink encephalopathy, and a mule deer with chronic wasting disease). For example, the incubation periods and lesion profiles seen in transmissions from six sheep with scrapie, collected since 1985, are shown in Figs 1a and 2a. Within the same timescale a further two sources of sheep scrapie failed to transmit to mice. In general, natural scrapie transmissions in our own laboratory and elsewhere have given variable results, probably reflecting variation in agent strain amongst the sheep sources[12,13].

At the time of writing, the transmissions of vCJD to mice have been in progress for 360 days. The RIII mice injected with all three vCJD sources have developed a progressive clinical disease very similar to BSE, with incubation periods in individual mice ranging from 288 to 351 days. The first signs were nervousness and hypersensitivity, followed by lethargy, weight loss, urinary incontinence and postural abnormalities. Excluding early intercurrent deaths, all RIII mice injected with vCJD have developed disease, with mean incubation periods up to a standard clinical endpoint of 304 ± 4, 306 ± 6 and 310 ± 4 days (±s.e.m.) for three sources (Fig. 1d), within but at the lower end of the range previously seen for BSE and related isolates[6] (Fig. 1b, c). Clinical signs are now apparent in some of the C57BL mice, an observation that is also consistent with the BSE pattern (see Fig. 1b). Diagnosis was confirmed for all clinically affected mice by the presence of vacuolar degeneration in
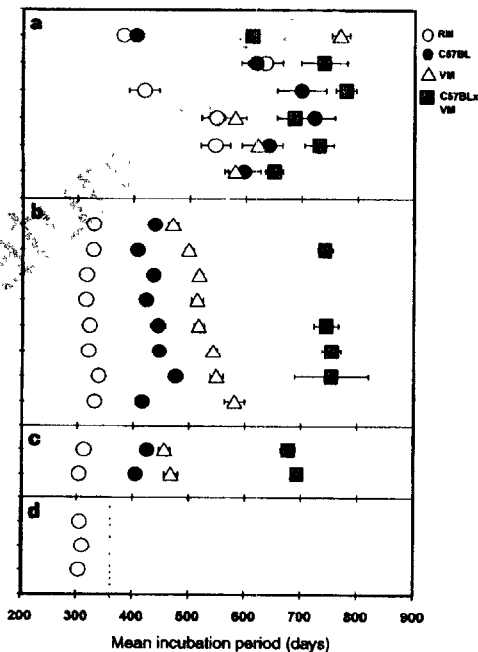
Figure 1 Incubation times in mice with spongiform encephalopathies Incubation periods in RIII, C57BL, VM and C57BL × VM mice in transmissions of: a, natural scrapie from six sheep; b, BSE from eight cattle; c, FSE from two cats; and d, three cases of vCJD C57BL × VM mice were not included in the first, third and fourth BSE transmission: missing symbols elsewhere indicate that no clinical disease was seen in these groups up to the natural lifespan of the mice The vertical dotted line in d shows the current time after challenge in experiments still in progress Data are mean ± s.e.m.
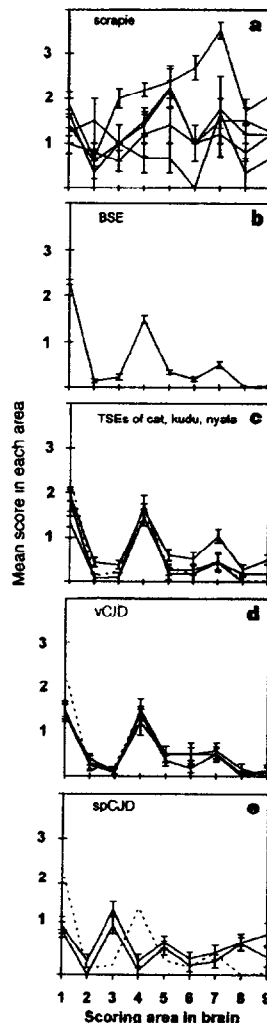
Figure 2 Lesion profiles for mice following transmission of spongiform encephalopathies. Lesion profiles are for RIII mice in transmissions of a, natural scrapie from five of the six sheep from Fig. 1a (n = 3–16 mice per group; no clinical disease was seen in this mouse strain in the sixth transmission); b, BSE from the first four cattle (n = 123, pooled data); c, FSE from two cats (n = 36, pooled data) and TSEs from a greater kudu and a nyala (n = 12 and 11); d, vCJD from three sources (n = 10, 12 and 16) and ●, spCJD from two sources, a farmer and a contemporary case (n = 8 and 9). The pooled BSE profile is shown as a dotted line in c-●. As white-matter vacuolation was not a prominent feature in any of these transmissions, only the grey-matter lesion profiles are shown Vacuolation was scored on a scale of 0–5 in the following scoring areas: 1, dorsal medulla; 2, cerebellar cortex; 3, superior colliculus, 4, hypothalamus; 5, thalamus; 6, hippocampus; 7, septum; 8, retrosplenial and adjacent motor cortex; and 9, cingulate and adjacent motor cortex. Data are mean ± s.e.m.
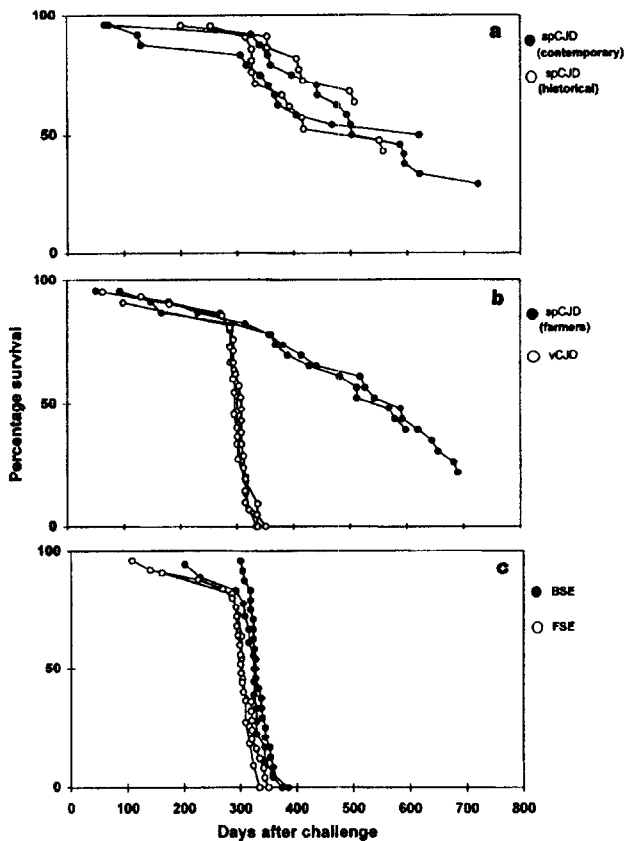
Figure 3 Survival curves for mice following transmission of spongiform encephalopathies. Survival curves are for female RIII mice in transmissions of: **a**, spCJD from cases with no known occupational exposure to BSE; **b**, spCJD from two farmers and vCJD from three sources; and **c**, BSE from the first two cattle sources and FSE from two cats. No distinction is made between mice dying with clinical signs of TSE infection and mice dying with intercurrent disease. Deaths up to 50 days after challenge, most of which were related to injection trauma, are excluded from the analysis.

the brain and for selected mice in all three experiments by the demonstration of relatively protease-resistant isoforms of PrP (PrP^{res}) in western blots of brain extracts and pathological accumulations of PrP in immunostained brain sections.

The neuropathology in clinically affected RIII mice with vCJD was also similar to that seen in RIII mice with BSE, consisting of a mild-to-moderate grey-matter vacuolation of the hypothalamus, medulla oblongata and septum, and a more severe vacuolation of the cochlear nucleus. Amyloid plaques were not a prominent feature of this pathology. The lesion profiles in RIII mice for the three sources (Fig. 2d) were very similar to each other and also to those in transmissions to RIII mice of BSE (Fig. 2b), TSEs of cats and exotic ruminants (Fig. 2c), and experimental sheep, goat and pig BSE[6,7], but differed markedly from those seen in transmissions from sheep with natural scrapie (Fig. 2a). Although results are so far only available for the RIII mouse strain, the striking similarity between vCJD and BSE in these mice, in terms of both incubation periods and pathology, is in itself strong evidence that the same strain of agent is involved in vCJD and BSE.

In contrast to the results with the vCJD sources, no clinical signs of neurological disease have yet been seen in any mice in the six transmissions of spCJD, although they have been in progress for between 600 and 800 days. Figure 3 shows survival curves for RIII

mice in these experiments, compared with survival curves in BSE, feline spongiform encephalopathy (FSE) and vCJD transmissions. No significant differences in median survival times were found between RIII groups challenged with the six spCJD sources, or between these groups and saline-injected controls. However, this does not indicate a failure to transmit spCJD, as vacuolar degeneration typical of TSE infection was seen in the brains of some mice dying with intercurrent disease in all six experiments, from about 400 days after challenge. This pathology has so far been seen in 30 of the 156 mice surviving beyond 500 days after injection for which brain was available for histopathological scrutiny. No such changes have been seen in the control mice of any age in this set of experiments, or in mice of the same strains injected with human brain homogenates from patients with amyotrophic lateral sclerosis or laryngeal carcinoma in a previously study[14]. Western blot and immunohistochemical analyses have demonstrated the accumulation of PrP^{res} in selected brains showing vacuolar pathology, confirming successful transmission of a TSE from all six spCJD sources.

A full analysis of the pathology in recipient mice in spCJD transmissions will be presented when these experiments are complete, but already several points can be made. Vacuolar degeneration has been seen in all four mouse strains. This pathology differs in severity between individual mice, but shows a consistent pattern between mouse strains and between spCJD sources. The earliest pathology is seen consistently in the superior colliculus and olfactory tract. In brains showing more widespread vacuolation there is also prominent involvement of the cerebral cortex, thalamus, hypothalamus, caudate nucleus and optic tract, a distribution unlike that seen in BSE transmissions to mice. As an illustration, Fig. 2e shows the lesion profiles for RIII mice killed with intercurrent disease or culled between 500 and 750 days after challenge with two spCJD sources (a farmer and a contemporary case). Although these profiles are not based on animals at the clinical endpoint of the disease, they clearly show a similarity in lesion distribution between the two sources of spCJD, and a difference between these sources and vCJD or BSE, particularly in scoring area 3, the superior colliculus. The results of these transmissions therefore provide no evidence of a link between CJD in dairy farmers and BSE.

A series of transmissions to mice of spCJD and familial human TSEs associated with mutations in the PrP gene have been reported in Japan[15]. Although different mouse strains were used in the Japanese series, the results for transmissions of spCJD from 129-methionine sources were broadly similar to ours in that transmission was achieved from all sources and mean incubation periods in recipient mice were long (573–863 days)[15]. Transmissions of the familial TSE Gerstmann–Straussler–Scheinker syndrome (GSS) were achieved from only one-third of the sources tested, but the mean incubation periods in successful transmissions were relatively short (237–517 days)[15]. Although some of these incubation periods were quite close to our results for vCJD in RIII mice, the pathology in mice with GSS was strikingly different as it included a prominent vacuolation of white-matter tracts[16]. The Japanese workers also reported the transmission of another human familial TSE, fatal familial insomnia (FFI), with a mean incubation period of 455 days in recipient mice[17]. The pathology in mice with FFI was indistinguishable from that in mice with spCJD in the Japanese series, apart from there being a more pronounced involvement of the thalamus.

Our results highlight several fundamental features of the TSEs previously established using experimental isolates[3,4]. The consistency in transmission properties shows that the agent must interact with genetic factors in the host to control the timing and neuropathology of the disease with extraordinary precision. Different strains of agent (spCJD, vCJD) can be isolated from hosts with the same PrP amino-acid sequence (in this case, patients with the 129-methionine genotype) but, conversely, the same strain of agent can

be detected in hosts with different PrP sequences (so far the BSE 'signature' has been seen in transmissions from eight different species[6]). This clearly indicates that TSE agents carry some form of information that specifies strain-specific properties, but the molecular basis of this information is still a matter for speculation[4].

It has been reported that vCJD can be distinguished from spCJD by the relative prominence of differently glycosylated forms of PrP[res] and the molecular size of the unglycosylated form[18]. Samples from dairy farmers with CJD have given glycoform ratios resembling those from other cases of spCJD[19]. A similarity in glycoform patterns between vCJD and BSE has been presented as evidence of a link between the two[18]. However, a 'BSE-like' glycoform pattern has also been seen for experimental scrapie isolates that are unrelated to BSE[20] and for FFI in humans[21]. Therefore, although the analysis of PrP diversity provides a useful supplement to strain typing in mice, it is premature to draw conclusions concerning causative links between TSEs in different species on the basis of glycoform-ratio analysis alone. A full analysis of glycoform patterns in the present series of transmissions will be reported in due course.

In conclusion, strain typing based on transmission to mice has shown: that vCJD is caused by the same strain of agent that has caused BSE, FSE and TSEs in exotic ruminants; that vCJD is distinguishable from spCJD; and that CJD in two dairy farmers is of the spCJD type and is not linked to the causative agent of BSE. Epidemiological surveillance continues to indicate that vCJD is a new condition occurring almost exclusively in the UK. Our transmission studies, in combination with the surveillance data, provide compelling evidence of a link between BSE and vCJD. □

Methods

CJD inocula. The CJD challenge experiments were the first to be undertaken within a new category 3 containment facility at the Neuropathogenesis Unit in Edinburgh, in an environment in which no TSE-infected materials had been handled previously. New dedicated glassware and instruments were autoclaved at 136 °C for 1 h before use. Brain samples for transmission were collected, as far as possible, from areas showing maximum pathology, and stored at −20 °C. Samples were homogenized at 10% (w/v) concentration in sterile physiological saline and stored at −20 °C. Before homogenization of each sample, sterile physiological saline was run through the homogenizer and other glassware and frozen for later inoculation of the appropriate control group. For injection, thawed homogenates were resuspended by being drawn repeatedly through a series of graded needles.

CJD transmissions. Three inbred mouse strains and one cross were challenged: C57BL and RIII (both of the Sinc[s7] or Prn-p[a] genotype), VM (Sinc[p7] or Prn-p[b] genotype), and the F[1] cross between C57BL and VM[22,23]. Groups of approximately 20 mice of each strain were injected by a combination of the intracerebral (20 μl) and intraperitoneal (100 μl) routes under halothane anaesthesia. For each transmission, six mice of each strain were injected with the appropriate saline sample by the same routes. Groups of uninjected control mice were also included. Mice were coded, examined daily throughout their lifespan, and formally scored for signs of neurological disease from 250 days after injection. Mice showing definite signs for two consecutive weeks were killed and incubation periods calculated as the interval between injection and this standard clinical endpoint[22]. All other mice were maintained to full lifespan, apart from small numbers culled at 700–750 days post-injection, to avoid loss of pathological material.

Histopathological and protein analysis. At post-mortem, a lateral third of each mouse brain was dissected aseptically and frozen at −20 °C for protein analysis and further passage. The remaining two-thirds of each brain was immersion fixed in 10% formol saline for 4 days, treated with 98–100% formic acid for 1 h to inactivate infectivity, and fixed in formol saline for a further 2 days. The brains were trimmed at standard coronal levels and paraffin embedded. Haematoxylin and eosin-stained sections 6 μm thick were prepared, randomly mixed with others from BSE, FSE, sheep scrapie and mouse-passaged scrapie transmissions, and coded for pathological assessment. Vacuolar changes were scored in nine grey-matter and three white-matter areas of brain for the construction of lesion profiles, as described[24]. PrP in brain

sections was immunostained using a polyclonal antibody to mouse PrP, 1A8 (ref. 25), according to a published protocol[26]. SDS–polyacrylamide gel electrophoresis and western blot analysis of brain tissue were used to confirm the presence of PrP[res] (ref. 18).

Animal TSE transmissions. The CJD transmissions were compared with transmissions of TSEs from cattle, sheep, domestic cats, a greater kudu and a nyala; the results of some of these animal TSE transmissions have been included in previous publications[5,6,8]. The design of these experiments was identical to that in the CJD transmissions, except that the source material from one cat, the greater kudu and the nyala was formol-fixed brain. Because transmissions from fixed tissues have resulted in prolonged incubation periods[5], probably owing to loss of titre, the incubation period and survival data from these three transmissions are not included in Figs 1 and 3. However, as the pathology in experimentally infected mice is unaffected by the dose of TSE challenge, lesion profile data from the kudu and nyala transmissions are included in Fig. 2.

Statistical analyses. Statistical analyses were performed using the software package Stata, version 5.0. Kaplan–Meier survival curves were plotted and differences in survival between mice inoculated with material from different sources were compared using the log-rank test[27]. Principal components analysis[28] was used to calculate summary measures of lesion profiles and to examine graphically the 'closeness' of the lesion profiles from different transmissions. This statistical analysis was in complete agreement with the subjective judgement of 'closeness' described in the text and will be documented in detail in a future publication.

1. Dickinson, A. G. & Meikle, V. M. H. Host-genotype and agent effects in scrapie incubation: change in allelic interaction with different strains of agent. Mol. Gen. Genet. 112, 73–79 (1971).
2. Fraser, H. & Dickinson, A. G. Scrapie in mice: agent-strain differences in the distribution and intensity of grey matter vacuolation. J. Comp. Pathol. 83, 29–40 (1973).
3. Bruce, M. E., McConnell, I., Fraser, H. & Dickinson, A. G. The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. J. Gen. Virol. 72, 595–603 (1991).
4. Bruce, M. E. Scrapie strain variation and mutation. Br. Med. Bull. 49, 822–838 (1993).
5. Fraser, H., Bruce, M. E., Chree, A., McConnell, I. & Wells, G. A. Transmission of bovine spongiform encephalopathy and scrapie to mice. J. Gen. Virol. 73, 1891–1897 (1992).
6. Bruce, M. et al. Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. Phil. Trans. R. Soc. Lond. B 343, 405–411 (1994).
7. Foster, J. D., Bruce, M., McConnell, I., Chree, A. & Fraser, H. Detection of BSE infectivity in brain and spleen of experimentally infected sheep. Vet. Rec. 138, 546–548 (1996).
8. Fraser, H. et al. Transmission of feline spongiform encephalopathy to mice. Vet. Rec. 134, 449 (1994).
9. Will, R. G. et al. A new variant of Creutzfeldt–Jakob disease in the UK. Lancet 347, 921–925 (1996).
10. Chazot, G. et al. New variant of Creutzfeldt–Jakob disease in a 26-year-old French man. Lancet 347, 1181 (1996).
11. Cousens, S. N. et al. Sporadic Creutzfeldt–Jakob disease in the United Kingdom: epidemiological data from 1970–1996. Br. Med. J. 315, 389–396 (1997).
12. Dickinson, A. G. in Slow Virus Diseases of Animals and Man (ed. Kimberlin, R. H.) 209–241 (North-Holland, Amsterdam, 1976).
13. Carp, R. I. & Callahan, S. M. Variation in the characteristics of 10 mouse-passaged scrapie lines derived from five scrapie-positive sheep. J. Gen. Virol. 72, 293–298 (1991).
14. Fraser, H., Behan, W., Chree, A., Crossland, G. & Behan, P. Mouse inoculation studies reveal no transmissible agent in amyotrophic lateral sclerosis. Brain Pathol. 6, 89–99 (1996).
15. Tateishi, J. Transmission of human prion diseases of rodents. Semin. Virol. 7, 175–180 (1996).
16. Tateishi, J., Ohta, M., Koga, M., Sato, Y. & Kuroiwa, Y. Transmission of chronic spongiform encephalopathy with kuru plaques from humans to small rodents. Ann. Neurol. 5, 581–584 (1979).
17. Tateishi, J. et al. First experimental transmission of fatal familial insomnia. Nature 376, 434–435 (1995).
18. Collinge, J., Sidle, K. C. L., Meads, J., Ironside, J. & Hill, A. F. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 383, 685–690 (1996).
19. Hill, A. F., Will, R. G., Ironside, J. & Collinge, J. Type of prion protein in UK farmers with Creutzfeldt–Jakob disease. Lancet 350, 188 (1997).
20. Somerville, R. A. et al. Biochemical typing of scrapie strains. Nature 386, 564 (1997).
21. Telling, G. et al. Evidence for the conformation of the pathological isoform of the prion protein enciphering and propagating prion diversity. Science 274, 2079–2082 (1996).
22. Dickinson, A. G., Meikle, V. M. H. & Fraser, H. Identification of a gene which controls the incubation period of some strains of scrapie agent in mice. J. Comp. Pathol. 78, 293–299 (1968).
23. Westaway, D. et al. Distinct prion proteins in short and long scrapie incubation period mice. Cell 51, 651–662 (1987).
24. Fraser, H. & Dickinson, A. G. The sequential development of the brain lesions of scrapie in three strains of mice. J. Comp. Pathol. 78, 301–311 (1968).
26. Farquhar, C. F. et al. in Transmissible Spongiform Encephalopathies (eds Bradley, R. & Marchant, B.) 301–313 (Commission of the European Communities, Brussels, 1994).
26. Bell, J. E. et al. Prion protein immunocytochemistry—UK five centre consensus report. Neuropathol. Appl. Neurobiol. 23, 26–35 (1997).
27. Kirkwood, B. R. Essentials of Medical Statistics (Blackwell, Oxford, 1988).
28. Mardia, K. V., Kent, J. T. & Bibby, J. M. Multivariate Analysis (Academic, London, 1979).