Early Hippocampal Synaptic Loss Precedes Neuronal Loss and Associates with Early Behavioural Deficits in Three Distinct Strains of Prion Disease

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Abstract

Prion diseases are fatal neurodegenerative diseases of the CNS that are associated with the accumulation of misfolded cellular prion protein. There are several different strains of prion disease defined by different patterns of tissue vacuolation in the brain and disease time course, but features of neurodegeneration in these strains have not been extensively studied. Our previous studies using the prion strains ME7, 79A and 22L showed that infected mice developed behavioural deficits in the same sequence and temporal pattern despite divergent end-stage neuropathology. Here the objective was to address the hypothesis that synaptic loss would occur early in the disease in all three strains, would precede neuronal death and would be associated with the early behavioural deficits. C57BL/6 mice inoculated with ME7, 79A, or 22L-infected brain homogenates were behaviourally assessed on species typical behaviours previously shown to change during progression and euthanised when all three strains showed statistically significant impairment on these tasks. A decrease in labelling with the presynaptic marker synaptophysin was observed in the stratum radiatum of the hippocampus in all three strains, when compared to control animals. Negligible cell death was seen by TUNEL at this time point. Astrocyte and microglial activation and protease resistant prion protein (PrP^res) deposition were assessed in multiple brain regions and showed some strain specificity but also strongly overlapping patterns. This study shows that despite distinct pathology, multiple strains lead to early synaptic degeneration in the hippocampus, associated with similar behavioural deficits and supports the idea that the initiation of synaptic loss is a primary target of the misfolded prion agent.

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Introduction

Prion diseases are fatal neurodegenerative diseases of mammals, including humans. The relationship between degeneration of particular neuronal circuits and the appearance of disease signs in mice, and indeed symptoms in humans, is largely unexplored. There is evidence to suggest that neuronal loss is a relatively late event and that this is preceded by synaptic loss [1,2]. Furthermore, there is evidence that reversing prion-associated toxicity in the early stages of disease, by conditional knockout of the normal cellular form of the prion protein, PrP^c, can return mice to an ‘asymptomatic’ state [3]. However, it remains unclear as to which neurons or neuronal pathways are first targeted and to what extent this is prion strain-dependent.

Different strains of murine prion disease have been characterised on the basis of brain tissue vacuolation pattern and incubation time [4,5]. The end stage pathologies of these strains are diverse and involve many different regions of the brain. The strains used in this study have distinct vacuolation profiles [4] indicating distinct neuropathology. The primary features of these strains are marked neuronal loss in the CA1 field of the hippocampus in ME7, prominent vacuolation of the white matter in 79A and cerebellar Purkinje cell death in the 22L strain. Despite these divergent end stage pathologies, mice infected with each strain show the same sequence of onset of behavioural deficits in a battery of tasks [6]. The sequence commences with a decline in spontaneous species-typical behaviours such as burrowing, glucose consumption and nesting, progressing to cognitive deficits and hyperactivity in open field assessment and later encompassing deficits in coordination, balance and muscle strength [1,6–9]. The similarity in the sequence of behavioural change in ME7, 79A and 22L indicates that there may be early common pathways that
were 10 mice in each scrapie strain and control group, all of
housed under standard light and temperature regimes. Food
become dysfunctional or degenerate and that certain
populations of neurons could be particularly vulnerable, but this
remains little investigated.

Hippocampal neuronal death has been demonstrated in
various prion strains but this is clearly a late stage
phenomenon [1,2,6] and is not found at 13 weeks post ME7
inoculation when behavioural deficits first appear [1]. However,
disturbances in synaptic proteins, loss of synapses and
dendritic alterations including the loss of spines, particularly in
the stratum radiatum of CA1, have been reported at 40-55%
disease duration [1,2]. Brain biopsies and post mortem tissue
from patients with prion disease have synaptic disorganisation
and loss, showing an accumulation of subcellular organelles,
dark synapses and a decrease in synaptophysin immunoreactivity [10–12]. Furthermore there is clear electron
microscopic evidence that presynaptic terminals begin to
degenerate and are subsequently surrounded or engulfed by
post-synaptic densities [13]. Given the convergence of data on
presynaptic terminal loss as an early event in the degenerative
process we investigated whether there is early synaptic loss
that is common to ME7, 79A and 22L strains, which are known
to show divergent end stage pathology. To investigate this,
mice were behaviourally monitored longitudinally until they
showed statistically significant deviations from control animals.
At this time, animals were euthanised and the tissue was
processed for markers of pre-synaptic terminals, microglia,
astrocytes, PrPSc and apoptotic cells in key areas known to
show pathology in one or more of the three strains examined in
this study.

Methods

Animals and stereotaxic surgery

Female C57BL/6 mice (Harlan, UK), n = 40, were group
housed under standard light and temperature regimes. Food
and water were available ad libitum, except during the glucose
test when a glucose solution was substituted for water. There
were 10 mice in each scrapie strain and control group, all of
which were used for behavioural testing and a minimum of
three from each group were used to assess pathology. All
procedures were carried out under a UK Home Office license
and in accordance with the Animals (Scientific Procedures) Act,
1986. Measures were taken to ameliorate the suffering of
animals in all experiments. The mice (14-20g) were
anaesthetised intraperitoneally with Avertin (2, 2, 2-
tribromoethanol) and positioned in a stereotaxic frame (David
Kopf Instruments, USA). The scalp was incised and the skull
exposed. Two small holes were drilled in the skull either side of
the midline to allow the bilateral injection of 1 µl of brain
homogenate (10% w/v in sterile PBS). Control animals were
injected with homogenate prepared from normal C57BL/6 mice
(normal brain homogenate, NBH-animals). Prion animals were
injected with homogenate prepared from C57BL/6 mice in the
terminal stages of scrapie of the ME7, 79A or 22L strain. The
ME7 strain tissue was generated in-house and 79A and 22L
strain tissue was obtained from the TSE Resource Centre,
Institute of Animal Health, UK. The injections were made
directly into the hippocampus with coordinates measured from
Bregma: anteroposterior -2.0 mm; lateral -1.7 mm; depth -1.6
mm. Injections were made with a 10 µl Hamilton syringe
adapted with a 26-gauge needle.

Behavioural testing

Mice were habituated to the burrowing and glucose tasks
and from week 10 post-injection were tested weekly. Plastic
cylinders, 20 cm long, 6.8 cm in diameter and closed at one
end were filled with 190 g of normal diet food pellets and
placed in individual cages. The open end was elevated 3 cm
above the base of the cage. Mice were placed into individual
cages in the late afternoon and the food remaining in the
cylinders after 2 hours was weighed and the amount of food
dispaced or burrowed was calculated. 5% glucose solution in
preweighed bottles was substituted for the drinking water. The
bottles and burrowing tubes were reweighed the next morning
and the amount of glucose drunk or pellets burrowed was
calculated.

Tissue processing and immunohistochemistry

Once statistically significant changes had been observed with
the behavioural tests (13 weeks after injection; see
results), animals were terminally anaesthetised with sodium
pentobarbitone and transcardially perfused with heparinised
saline followed by 10% formal saline. Brains were paraffin
embedded and 10 µm coronal sections through the septum,
dorsal hippocampus, thalamus, raphe and cerebellum were cut
on a microtome, dewaxed in xylene and rehydrated.
Immunohistochemistry was performed for microglia (IBA1,
Abcam, UK), glial fibrillary acidic protein (GFAP, Dako, UK),
synaptophysin (SY38, Chemicon, UK) and PrPSc (6H4,
Prionics, Switzerland). TUNEL staining kits were purchased
from Promega, UK. Biotinylated secondary antibodies, normal
sera, mouse-on-mouse (MOM) blocking kit and avidin-biotin
complex were from Vector Laboratories, UK. Immunohistochemistry was carried out by the avidin-biotin-complex method (ABC) method, with 0.015% v/v hydrogen
peroxide as the substrate and visualised with diaminobenzidine
(DAB). Primary antibody specific modifications are detailed
below.

Synaptophysin

Rehydrated sections were treated for 30 minutes with 0.2 M
boric acid, pH 9, 65 °C and incubated with 1% H2O2/PBS for 15
minutes to eliminate non-specific peroxidase activity. Sections
were washed in PBS and blocked with 10% normal horse
serum. Sections were incubated overnight with anti-
synaptophysin (1:2000) at room temperature, washed with PBS
and incubated with biotinylated horse anti-mouse secondary
antibody (1:200). The DAB reaction was carried out with the
addition of ammonium nickel sulphate (0.04% w/v) to enhance
the intensity. The sections were dehydrated and mounted in
Depex.

The density of synaptophysin staining was quantified by pixel
density analysis on digitally captured images using ImageJ
image analysis software (NIH, USA) using a similar method to
that which we previously published [1]. In the stratum under
test, areas of uniform staining were quantified by pixel density
analysis. A background reading was taken from the DG granule layer (the area of highest transmittance) as an internal standard and all other transmittances were subtracted from this. Four or five sections per animal from three animals per experimental and control groups were assayed. The data was expressed as a ratio of the transmittance in the stratum radiatum to the transmittance in the adjacent stratum lacunosum moleculare.

GFAP

Rehydrated sections were treated with 1% H2O2 to block endogenous peroxidase activity and microwaved at full power in 10 mM citrate buffer, pH 6, for 3 minutes followed by 5 minutes cooling and a further 5 minutes microwaving. Washed sections were incubated in 0.04% pepsin in 0.1 M HCl for 10 minutes. Sections were blocked with 10% normal goat serum followed by anti-GFAP, 1:2000 for 90 minutes at room temperature. Washed sections were incubated with biotinylated goat anti-rabbit (1:100). Sections were incubated with ABC and visualised with DAB. Sections were counterstained with haematoxylin, dehydrated and mounted in Depex.

The density of astrocytes was scored as 0, +, ++ or +++ in a blinded fashion with GFAP levels in NBH-animals in that region set at 0. Scoring was carried out in the lateral septum, dorsal part (LSD); the medial septum-diagonal band of Broca (MSDB), the dorsal hippocampal formation, the dorsal thalamus; dorsal raphe; median raphe; the simple lobule of the cerebellum. The results are presented relative to NBH scores.

IBA1

Rehydrated sections were treated as above to block endogenous peroxidase activity and for antigen retrieval with the addition of 0.1% Tween in the PBS wash solution. Sections were blocked with 10% normal horse serum and incubated overnight with anti-IBA1, 1:200 at 4 °C. Sections were incubated with biotinylated horse anti-goat (1:100). The antigen was visualised and the sections mounted as above.

The density of microglia was scored as 0, +, ++ or +++ in a blinded fashion with 0 equal to the widespread relatively uniform distribution of IBA-1-positive microglia seen in sections from NBH-animals. The scoring was carried out in the same structures as above and the results are presented relative to NBH scores.

PrPSc

Rehydrated sections were autoclaved at 121 °C for 15 minutes, washed in PBS and treated with 95% formic acid to destroy PrPSc. To avoid potential non-specific binding that can occur using a mouse monoclonal antibody, the sections were incubated with MOM blocking reagent, MOM diluent and the anti-PrP (6H4, 1:4000) was made up in diluent. Sections were incubated overnight at 4 °C. Washed sections were incubated with biotinylated anti-mouse IgG, (1:250) and the antigen was visualised and section mounted as above.

TUNEL

Rehydrated sections were pre-incubated in equilibrium buffer for 10 minutes at 37 °C and then with incubation medium for 2 hours at 37 °C, labelling fragmented DNA with fluorescein. The reaction was stopped with sodium citrate solution, blocked with 10% normal goat serum and subsequently incubated with biotinylated goat anti-fluorescein (1:200). Sections were then processed as above. Late stage ME7 hippocampal sections were processed alongside the early stage tissue as a positive control. TUNEL positive nuclei were counted in full coronal sections of the septum (distance from Bregma: 1.10 mm to 0.62 mm); dentate gyrus, hippocampus and thalamus (-1.46 mm to -1.82 mm); dorsal and median raphe (-4.36 mm to -4.48 mm); cerebellum (-5.40 mm to -5.68 mm).

Statistics

Behavioural data were compared by repeated measures two-way ANOVA to examine the effects of treatment group (ME7-, 79A-, 22L-, NBH-animals) and of time (10-13 weeks). Since the experiments were designed to terminate as soon as significant differences became apparent, all groups were also compared with NBH-animals with Bonferroni post-hoc tests. Ratio measurements of synaptophysin density were analysed by ANOVA with Bonferroni post-hoc tests. TUNEL counts were compared between groups by one-way ANOVA.

Results

Behavioural testing

The aim of this study was to compare neuropathological changes in the three strains once statistically significant changes on species typical behaviours had been observed in all three. The results of the burrowing and glucose tests were comparable to that seen in our previous studies [1,6]. When NBH-animals and the animals inoculated with each of 3 prion strains were compared on burrowing (Figure 1a) and glucose consumption (Figure 1b), similar patterns were observed for all three strains, with slightly differing temporal characteristics. We performed repeated measures two-way ANOVA on burrowing data, with strain (NBH, ME7, 79A and 22L) as the between subjects factor and time as the within subjects factor. This revealed main effects of time (F=49.93, df 3,108, p<0.0001) and an interaction of these factors (F=5, df 3,108, p<0.001) and an interaction of these factors.
Synaptic Loss in Prion Disease Strains

murine prion disease [1–3,5,6]. Micrographs of the laminar structure are shown for NBH (Figure 2a), ME7 (2b), 79A (2c) and 22L (2d). The full laminar structure of the hippocampus in an NBH-animal can be seen at lower power in Figure 2e, demonstrating the high density of presynaptic terminals in the stratum radiatum and stratum oriens of CA3 and CA1. This corresponds to the mossy fibre projection from the granule cells in the dentate gyrus to the pyramidal neurons in CA3 and the Schaffer collaterals from CA3 to CA1 respectively. At 13 weeks post-injection, there is disruption of the laminar structure in the CA1 region of the hippocampus and a reduction in the density of the synaptophysin staining in the stratum radiatum of all experimental groups when compared to NBH animals (Figure 2a vs. 2b-d). This appeared to be most severe in 79A and most variable in 22L but was present in all prion diseased animals. Pixel density analysis of synaptophysin labelling revealed that the ratio of transmittance in the stratum radiatum relative to the stratum lacunosum-moleculare is significantly decreased in ME7 (p<0.01), 79A (p<0.01) and in 22L (p<0.05) when compared to NBH-animals (Figure 2f; Bonferroni post-hoc comparisons to NBH after a significant one way ANOVA).

Astrocytes

In general, there was an increase in the density of astrocytes in selected brain regions in all three groups of prion-infected animals and the level of increase did show some strain specificity. GFAP positive astrocytes are present in the septal region of NBH-animals, however the number of astrocytes with detectable levels of GFAP has massively increased in the MSDB of prion infected animals (Figure 3a vs. 3b-d). Particularly in the 79A- and 22L-animals, this increase clearly highlights the border between the MSDB and the adjacent GFAP-negative shell of the nucleus accumbens (Figure 3c & 3d). Increased numbers of GFAP-positive astrocytes were also present in the dorsal part of lateral septum, which receives a projection from the hippocampus. In NBH-animals, GFAP positive astrocytes are detectable in the hippocampus and the dentate gyrus, whereas in the dorsal thalamus and the overlying cortex, astrocytes can only be seen around blood vessels (Figure 3e). In ME7- and 79A-animals, the hippocampus and the DG was a site of GFAP upregulation (Figure 3f & 3g). There was GFAP-positive astrocytosis in the hippocampus of 22L animals although this was more limited and more variable that in ME7 and 79A (Figure 3h). There was a dramatic increase in detectable levels of GFAP in the dorsal thalamus in all three strains compared to NBH-animals (Figure 3e vs. 3f-h), however it can be seen that in the ME7-animals there are some thalamic midline nuclei that do not have GFAP upregulation. In the dorsal and median raphe of NBH-animals, the only astrocytes with detectable levels of GFAP are around blood vessels (Figure 3i), while the dorsal and median raphe in all three prion strains showed an increase in GFAP positive astrocytes, with the greatest increase in the 22L-animals (Figure 3j–l). At 13 weeks, the level of GFAP positive astrocytes in the simple lobe of the cerebellum is similar in all four groups (Figure 3m–p). In the 22L-animals there is no or only weak up-regulation of GFAP in the vicinity of the Purkinje neurons, a cell population that is severely affected at late stage

Figure 1. Behavioural analysis of the prion-diseased and control animals. All three strains of prion disease agent caused behavioural deficits with a similar temporal sequence. a) In a food pellet-burrowing task, the amount (weight, g) burrowed after two hours was plotted against weeks post-inoculation. b) In the glucose consumption test, the amount of 5% glucose solution drunk overnight, when singly housed, was measured. Polydipsia was established but consumption subsequently began to decline significantly in all strains with respect to NBH (n = 10 animals per strain, *P < 0.05, **P < 0.01, ***P < 0.001, Bonferroni post hoc test after significant effects in repeated measures two-way ANOVA).

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(F=3.95, df 9.108, p<0.0005). Bonferroni post-hoc tests showed that differences between NBH and 22L reached significance at 11 weeks (p<0.05), ME7 was significantly different from NBH from 12 weeks (p<0.05) and 79A was significantly different to NBH from 13 weeks (p<0.01). NBH animals continued to drink large amounts of glucose solution (>15 ml).

Synaptophysin

Synaptophysin staining identifies presynaptic terminals and has been used previously to demonstrate synaptic loss in...
in this strain (Figure 3p). The density of GFAP positive astrocytes in the structures of interest was scored as 0, +, ++ or +++ and the results are presented relative to the scores from NBH-animals (designated 0) (Table 1).

**Microglia**

IBA-1 is constitutively expressed in microglia. In NBH-animals, immunohistochemistry for IBA1 showed microglia sparsely distributed throughout the brain. We observed a large increase in the numbers of microglia in a number of regions in the prion-diseased animals. The lateral septum, which receives a direct projection from the hippocampus showed significant increases in IBA-1-positive microglia in the ME7- and 79A-animals (Figure 4b & 4c) and more limited activation in 22L (Figure 4d). This is consistent with the more robust microglial activation in the hippocampus of ME7 and 79A (Figure 4f & 4g) with respect to the more limited labelling in 22L (Figure 4h). Microglia in prion strains show a more condensed morphology with respect to the ramified morphology in NBH animals (insets). In the median raphe, there was an increase in the number of microglia in all prion animals (Figure 4j–l) although this increase was most prominent in the 79A- and 22L-animals (Figure 4k & 4l). In the cerebellum, typified by the simple lobule, there was no increase in the density of microglia in the ME7- and 79A-animals compared to NBH-animals (Figure 4m vs. 4n & 4o). There was very limited and variable increase in the number of microglia in 22L-animals (Figure 4p). The density of microglia was scored as 0, +, ++ or +++ and the

![Figure 2. Hippocampal synaptophysin in prion-diseased and normal brain homogenate-inoculated animals.](image-url)
results were presented relative to the scores from NBH-animals (designated 0) (Table 1).

**PrP**

In all prion disease animals, at 13 weeks after injection, there were deposits of PrP^Sc protein present in the regions of the brain examined (Figures 5b–d, 5f–h, 5j–l & p) and no deposition in the control animals (Figures 5a, 5e, 5i & 5m). In the septum of 79A-animals, there is prominent deposition of PrP^Sc in the MSDB and the lateral septum (Figure 5d). In the hippocampus, there is prominent deposition of PrP^Sc.
deposition in the polymorphic layer of the dentate gyrus and the stratum lucidum of CA3 in the hippocampus for all three strains and granular deposition in the other layers of the dentate gyrus (Figure 5f–h). In contrast to the ME7- and 79A-animals, the CA1 region of the 22L-animals has a sparse deposition of PrP\textsuperscript{Sc} in the stratum radiatum and stratum oriens (Figure 5h) compared to its marked deposition in the dentate gyrus of these animals. PrP\textsuperscript{Sc} deposition in the dorsal and median raphe is found in all three strains (Figure 5j–l) and the deposition is very extensive in the 22L-animals (Figure 5l). In the simple lobe of the cerebellum, the ME7- and 79A-animals are indistinguishable from control animals (Figure 5m vs. 5n & 5o). In 22L-animals there is a small and variable amount of deposition around the Purkinje neurons (Figure 5p). A summary of these assessments is shown in table 1.

### Technical considerations

One might argue that the injection of 1\(\mu\)l of 10% w/v infected brain homogenerate into the dorsal hippocampus might be predicted to induce hippocampal pathology and dysfunction. However, it is clear that this dysfunction does not occur until 10–13 weeks post inoculation and is not seen in NBH-animals, so cannot be a result of surgery-induced hippocampal injury. Moreover, prion infectivity is well distributed through the brain parenchyma in this time. Many protocols still administer prion-infected homogenate no more precisely than ‘intracerebrally’ or ‘intrahippocampally inoculated’. These common protocols exert little control over where the inoculum goes and leads to minimal direct damage to the hippocampus and surrounding structures. However, more precise protocols offer a greater degree of standardisation. The intra-hippocampal injection procedure leads to minimal direct damage to the hippocampus and animals displaying ‘clinical signs’ using this surgical protocol developed pathology [6] faithful to the published profiles documented after homogenate injection into the frontal cortex [4,5]. Clearly intraperitoneal inoculation induces substantially different survival times and pathological profiles compared to intracerebral inoculation [15] and intracerebral injections in the cerebellum, close to presumed brainstem ‘clinical target areas’ [19,20], can also produce significantly different patterns of PrP\textsuperscript{Sc} deposition and vacuolation [21] indicating that site of disease initiation can influence disease course. However, our protocol does induce disease progression, survival times and late stage pathology consistent with other, less precise,
forebrain-initiated surgical protocols. Furthermore, robust behavioural deficits in the burrowing task and the glucose consumption test are seen in animals with striatal or cerebellar injection at these early time points with no overt clinical symptoms [16].

The results show significant areas of similarity between these strains as well as features that distinguish them from each other. As previous terminal stages studies would have predicted, there is some cerebellar tropism in the 22L strain that is not obvious in ME7 and 79A strains, and indeed 22L shows somewhat weaker synaptic loss, microglial activation and PrPSc deposition in the CA1 of the hippocampus with respect to the dentate gyrus/CA3 region. However, that 22L can produce CA1 synaptic loss is significant, and this has now been confirmed by electron microscopy studies [22]. On the whole 79A produces rather similar early pathology to ME7. As we reported in terminal stages of these 3 strains [6], it is once again evident from microglial, astrocytic and PrPSc

Figure 4. IBA1 positive microglia increase and have an activated morphology in prion animals. a–d) Compared to the sparse, uniform distribution of microglia in NBH animals, the lateral septum, dorsal part, shows an increased expression in the prion strains (4a vs. 4b-d). e–h) CA1 of the hippocampus shows a large increase of microglia with activated morphology (insets) in the ME7 and 79A strains, with a more modest increase in the 22L strain (4e vs. 4f-h). i–l): The median raphe has increased microglia in all strains (4i vs. 4j-l). m–p) microglia levels were similar in all treatment groups. Scale bars = 100 µm.

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immunolabelling that the thalamus is significantly affected in all 3 strains and the emergence of this pathology, which cuts across several different ventrobasal nuclei of the thalamus, requires further study. Combining the data arising from this study with prior studies with different strains and different sites of inoculation, it appears that these multiple strains can produce limbic system and thalamic pathology given appropriate exposure to the inoculum. Though key clinical target areas have not been definitively identified, it appears that inoculation close to the brainstem will lead to death of the animal before pathology can become evident in some of the regions identified in this forebrain-initiated study.

Figure 5. PrPSc deposition is widespread at 13 weeks in the prion-infected animals, but there are strain specific differences. a–d) PrPSc is deposited in a granular form or in large, dense plaques. There is no PrPSc deposition in the control animals. e–h) The dentate gyrus and the hippocampus have a high PrPSc load in all three strains and none in the NBH animals. There is a strain difference in the CA1 field with deposition in the 22L strain predominantly confined to the stratum oriens and radiatum. i–l) PrPSc is deposited in the median and dorsal raphe, especially in the 22L strain. m–p) In the simple lobule of ME7 and 79A animals PrPSc deposition is indistinguishable from that seen in NBH-animals. There is a small amount of PrPSc around the Purkinje cells of the 22L mice. Scale bars = 500 µm a–l, 100 µm m–p.

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Synaptic loss preceding neurodegeneration

The most consistent finding of this study was that the behavioural deficits were associated with a decrease in synaptophysin immunostaining in the CA1 field of the hippocampus in all three prion strains. Of particular note is the synaptic loss in the 22L strain, which does not have CA1 pyramidal cell death even at the later stages of the disease [6]. This advances the previous finding that behavioural changes and hippocampal synaptic loss in ME7 animals occurs several weeks prior to neuronal cell death [1]. Jeffrey and colleagues noted the first axon terminal degeneration and synaptic loss at 34-42% of the incubation period (IP) in ME7 in C57BL/6J x VM/Dk mice, but no cell death in CA1 until 72% IP [2]. In the current study, the first behavioural deficits were found at 11 weeks and both burrowing deficits and decreased glucose consumption were present in all three strains by 13 weeks. At this time all animals were euthanized. Synaptic loss was present in all three strains and hippocampal or limbic system neuronal loss was absent in all three strains. Although a decrease in synaptic proteins such as synapsin, SNAP-25 and synaptophysin has been demonstrated in post mortem CJD cerebral cortex and cerebellum [10,23,24] and in the RML mouse model [25], these analyses were undertaken at a time when there was extensive neuronal death and thus severe synaptic loss would be expected. The current study demonstrates clearly that, if exposed to the prion infectivity, all three strains ME7, 79A and 22L show robust synaptic loss in the hippocampus and early behavioural deficits. It has been shown that hippocampal lesions have a profound affect on the burrowing behavioural assay [26] and while we show a clear correlation between hippocampal synaptic loss and the onset of a burrowing deficit in these prion strains we cannot exclude the possible involvement of other brain structures. The thalamus, in particular, is clearly affected in all three strains [6]. Glucose consumption could be predicted to involve other brain structures such as the lateral hypothalamus [27], and some authors have reported vacuolation in this region in ME7 and 79A strains [28]. Nonetheless possible involvement of multiple brain structures, in neurofilament heavy chain-Cre mice inoculated with the RML-strain of prion disease, it has been demonstrated that synaptic loss can be reversed by switching off neuronal expression of PrPc and this also reverses early behavioural/cognitive dysfunction [3]. Furthermore, prevention of neuronal apoptosis by induction of prion disease in Bax/-/- mice had no impact on time to onset of clinical signs or time of death and had no impact on synaptic loss [29]. Collectively these data suggest that synaptic loss is indeed an early component of prion pathology that is correlated with behavioural/clinical changes and our current data indicate that the described synaptic changes are likely to occur in many different prion strains after intracerebral inoculation.

Synaptic degeneration has also been identified in other CNS neurodegenerative disorders. Synaptic loss is regarded as the best correlate of cognitive decline [30] in Alzheimer’s disease and, congruent with this, synaptic loss is present but less prominent in mild cognitive impairment (MCI) and in transgenic AD models [31–33]. Synaptic loss has also been documented as an early event, preceding most neuronal death in Huntington’s disease and in motor neuron disease, correlating with the deterioration of mental and motor functions [34–36]. Thus synaptic loss is a key feature of many chronic neurodegenerative diseases and occurs before neuronal death in many disease states. Little is known about the mechanisms by which presynaptic terminals degenerate in any of these conditions and the robust and reproducible synaptic loss in the stratum radiatum in these prion strains presents a tractable model to study aspects of the cellular and molecular mechanisms that underpin this process.

Mechanisms of synaptic loss

Biochemical studies of the hippocampus of ME7-animals showed that proteins of the presynaptic terminal compartment were the first to decline and in particular those of the synaptic vesicles [37]. There are also reports of significant cortical dendritic spine alterations in the RML strain [38]. However, recent electron microscopy studies with ME7 animals have revealed that the loss of the integrity of the synaptic vesicles is the first apparent morphological feature of synapse degeneration in the stratum radiatum (Siskova et al., 2009). The electron dense pre-synaptic element remains in close apposition to the postsynaptic density of dendritic spine which becomes progressively wrapped around degenerating presynaptic elements [13]. Identical profiles are seen in the stratum radiatum of 22L-animals at 12 weeks after disease initiation [22]. Simultaneously, synaptic swelling/hypertrophy occurs in apparently intact presynaptic terminals [39], which may be a precursor to, or a mechanism to compensate for, synaptic loss.

PrPc is highly concentrated in presynaptic terminals where it co-localises with synaptophysin and is highly prevalent in brain structures associated with synaptic plasticity [40–43] and PrPSc knockout mice show deficiencies in GABAergic receptor mediated fast inhibition and impaired long-term potentiation (LTP) [44]. Those data suggest that the loss of normal PrPc function may contribute to synaptic dysfunction and ultimately to neuronal loss. However, it has been shown that blocking synaptic activity in the ME7 model, using botulinum neurotoxin A, does not induce any additional or more rapid synaptic loss in these animals [45]. Furthermore there are studies to show that PrPSc generated by astrocytes in the absence of neuronal expression of PrPc still leads to neuronal degeneration [46]. There remains no clear understanding of the physiological function of PrPc, but the accumulation of oligomeric aggregates of the aberrant PrPSc [47] initiates neuronal degeneration at the pre-synaptic terminal in a number of different prion strains by mechanisms that have yet to be unravelled.

Conclusion

Synaptopathy in the hippocampal formation is common to three strains of prion disease upon intra-hippocampal inoculation and is an early pathologcal feature of the disease. Since these features occur in association with behavioural changes but significantly before neuronal death, this indicates that synaptic loss is a key neuropathological feature of multiple strains of prion disease. Thus, these prion disease models offer
Satisfactory routes into elucidation of molecular and cellular events underpinning synaptic loss. Further studies of these models may provide insights into synaptic degeneration that may be exploited in multiple disease states.

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