Loss of Perineuronal Net in ME7 Prion Disease

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Abstract
Microglial activation and behavioral abnormalities occur before neuronal loss in experimental murine prion disease; the behavioral changes coincide with a reduction in synaptic plasticity. Because synaptic plasticity depends on an intact perineuronal net (PN), a specialized extracellular matrix that surrounds parvalbumin (PV)-positive GABAergic (γ-aminobutyric acid [GABA]) inhibitory interneurons, we investigated the temporal relationships between microglial activation and loss of PN and PV-positive neurons in ME7 murine prion disease. Anesthetized C57Bl/6J mice received bilateral intracerebral microinjections of ME7-infected or normal brain homogenate into the dorsal hippocampus. Microglial activation, PrPSc accumulation, the number of PV-positive interneurons, and Wisteria floribunda agglutinin-positive neurons (i.e. those with an intact PN) were assessed in the ventral CA1 and subiculum at 4, 8, 12, 16, and 20 weeks postinjection. Hippocampal areas and total neuron numbers in the ventral CA1 and subiculum were also determined. Loss of PN coincided with early microglial activation and with a reduction in synaptic plasticity. No significant loss of PV-positive interneurons was observed. Our findings suggest that the substrate of the earliest synaptic and behavioral abnormalities in murine prion disease may be inflammatory microglia-mediated degradation of the PN.

Key Words: CNS inflammation, Interneuron, Microglia, Pathogenesis, Perineuronal net, Prion disease.

INTRODUCTION
The prion diseases are chronic neurodegenerative disorders that affect human beings (e.g. Creutzfeldt-Jakob disease [CJD]) and animals (e.g. sheep scrapie, bovine spongiform encephalopathy in cattle) (1). The causative agents of these diseases are considered to be an aberrant protein, designated PrPSc (scrapie-associated prion protein), which derives from PrPC (normal cellular prion protein) (2). The pathologic hallmarks of end-stage disease are similar in human, animal, and experimental models of the disease and include the deposition of PrPSc, vacuolation, astrocytosis, microglial activation, and neuronal loss. The study of the early pathologic and functional abnormalities in prion disease has been facilitated by the use of experimental models. Using a murine model, we and others have described the sequence of neuropathologic (e.g. microglial activation, recruitment of cytotoxic T lymphocytes, synaptic loss, and degradation of the perineuronal net), electrophysiologic (e.g. changes in synaptic plasticity), and behavioral changes that occur during a 24-week incubation period (3–6). Briefly, there is a progressive increase in the number of activated microglia and reactive astrocytes that begins approximately 8 weeks postinjection (p.i.). From 12 p.i. weeks onward, there is reduction in synaptic plasticity (long-term potentiation [LTP]) in ventral hippocampal brain slices and a failure of prion-affected mice to perform hippocampus-dependent behavioral tasks. Neuronal cell loss is not apparent in the pyramidal cell layer of the dorsal hippocampus until 20 weeks p.i. There then follows a period of rapid clinical decline characterized by motor incoordination, weight loss, and a reduction in mobility between 20 and 24 weeks p.i.

Reduction in LTP may underlie the cognitive decline that we previously reported in murine prion disease (5). The physiologic expression of hippocampal LTP depends on the integrity of a specialized region of the extracellular matrix (ECM), termed the perineuronal net (PN). The PN is sandwiched between glial processes and the neuronal surface and surrounds synapses (7, 8). In the cerebral cortex, the PN is predominantly associated with parvalbumin (PV)-positive GABAergic (γ-aminobutyric acid [GABA]) inhibitory interneurons (9, 10). In the hippocampus, the PN is most abundant around GABAergic interneurons within or around the pyramidal cell layer of the hippocampus proper, subiculum, and presubiculum, but also distributed more diffusely in the neuropil of the CA2/CA3 transition region and the dorsal and ventral presubiculum and subiculum (11). The PN is composed of chondroitin sulfate proteoglycans (CSPGs), including the lecticans (neurocan, versican, aggregan, and brevican), the matrix-associated proteoglycan phosphocan, and cell surface–associated proteoglycans such as neuroglycan C and NG2. Constituents of the PN associate noncovalently with more widely distributed components of the ECM, including hyaluronan, tenascin, laminin, and fibronectin, and with cell-surface adhesion molecules. The glycosaminoglycan side chains of the CSPGs within the PN bind to other ECM constituents, cell-surface receptors, and...
many cell signaling molecules. The composition of the ECM is regulated by neural activity (12) and by matrix metalloproteinases (MMPs), and this modulates synaptogenesis and synaptic plasticity (13). Particular components of the PN play a direct role in LTP. For example, tenascin and brevican knockout mice show a reduction in LTP (14, 15), and we observed a similar reduction in brain slices from prion-affected mice (6). A loss of PN has been reported in human and experimental CJD (16, 17); in the latter, the loss occurs at early stages of the disease and precedes neuronal degeneration.

The mechanisms of PN degradation in prion disease and whether the degradation accounts for the reduction in hippocampal LTP and the development of behavioral abnormalities are unclear. In the present study, we used the ME7 murine prion disease model to explore the hypotheses: i) that the degradation of the PN is related to microglial activation and ii) that the timing coincides with the onset of the electrophysiologic and behavioral abnormalities which we described previously in this model. We have also investigated the relationship between degradation of the PN and the integrity of PV-positive interneurons that it surrounds.

MATERIALS AND METHODS

Mice

All studies were performed in female C57BL/6J mice (Harlan, Bicester, UK) aged 6 to 8 weeks in accordance with the Animals (Scientific Procedures) Act of 1986. Mice were housed in transparent plastic cages in a room at 20°C, 12-hour light, 12-hour dark cycle, with free access to food and water. After a 1-week acclimatization period, the mice were anesthetized (using an intraperitoneal injection of Hypnorm (11%; Jansen-Cilag, High Wycombe, UK) and Hypnovel (20%; Roche, West Sussex, UK) at a dose of 0.1 and 0.05 ml/10 g, respectively) and positioned in a stereotaxic frame. They received bilateral injections of 1 μl of 10% wt/vol of ME7 brain homogenate (derived from brains of terminally affected, previously ME7-injected mice) through a terminal dose of anesthetic and perfused via the transcardic route with heparinized saline followed by 10% formalin. The brains were removed, postfixed in formal saline, and processed for paraffin histology (n = 6 for the NBH- and ME7-injected groups at each time point, total = 60).

Immunohistochemistry

Horizontal sections were cut at 4 μm at the level of the ventral hippocampus. Immunohistochemistry was performed on dewaxed paraffin sections, with antibodies to PV (monoclonal anti-PV antibody; Sigma, Gillingham, UK) and PrPSc (monoclonal antibody 6H4; Prionics, Zurich, Switzerland). All sections were incubated for 30 minutes in methanol containing 3% hydrogen peroxide to eliminate endogenous peroxidase activity. To demonstrate PrPSc, sections were autoclaved (in distilled H2O for 15 minutes at 121°C) and then incubated in 98% to 100% formic acid for 5 minutes. No pretreatments were required to demonstrate PV interneurons. The sections were rinsed thoroughly and incubated in blocking serum for 30 minutes at room temperature and overnight at room temperature with the primary antibodies: 6H4 (1:2000) or PV (1:2000). The sections were then incubated with biotinylated secondary antibodies for 20 minutes at room temperature. Bound primary antibodies were detected using the avidin-biotin peroxidase method (Vectastain Elite ABC; Vector Laboratories, Peterborough, UK), with diaminobenzidine hydrochloride (DAB) as the chromogen. Using a copper sulfate solution, DAB staining was enhanced. Sections were lightly counterstained with hematoxylin.

Lectin Histochemistry

Histochemistry with biotinylated Lycopersicon esculentum agglutinin (tomato lectin) (Sigma) was used to demonstrate microglia (18) and with Wisteria floribunda agglutinin (WFA) (Sigma) to demonstrate the PN (19). Endogenous peroxidase was blocked as described in the previous sentences. Different pretreatment protocols were used for histochemistry with tomato lectin: three 10-minute immersions of the sections in Tris-buffered saline (0.05 mol/L of Tris base, 0.9% NaCl, pH 7.6) with 1% triton containing 0.1 mmol/L: magnesium, manganese, and calcium chlorides, and WFA sections were incubated in citrate buffer at pH 6 and microwaved twice to boiling point, with intervals of 5 minutes. Sections were incubated either with tomato lectin (1:20; overnight at 4°C) or with avidin (15 minutes) and biotin (15 minutes; avidin-biotin blocking kit; Vector Laboratories) to block endogenous biotin activity, and then in WFA (1:32000; 1 hour at room temperature). Lectin binding was visualized using DAB as the chromogen, as outlined in the previous sentences, and DAB staining enhanced using a copper sulfate solution. Sections were lightly counterstained with hematoxylin.

Double-Fluorescent Staining

Double-fluorescent staining was performed on selected sections at each experimental time point. The pretreatments and preincubation with blocking serum were as described in the previous sentences. Sections were incubated overnight and at room temperature with a mixture of PV antibody and WFA. The sections were then incubated for 30 minutes, at room temperature, with a mixture of fluorescein isothiocyanate-conjugated avidin D (1:100; Vector Laboratories) to label bound biotinylated WFA and Texas Red anti-mouse (1:100; Vector Laboratories) to label bound anti-PV antibody. Sections were mounted in Vectashield medium (Vector Laboratories).

Histologic Analysis

For all histologic analyses, sections were examined by observers blinded to the experimental group and time point. Histometrix software (Kinetic Imaging, Nottingham, UK),
driving a Leica DM (Wetzlar, Germany) microscope with a motorized stage was used to quantify labeling of microglia and PrPSc. On a 10× objective image, the ventral CA1 or subiculum was interactively outlined, and within the outlined area, the software was then used to make an automated unbiased selection of seven 40× objective fields in which the percentage (%) of the area labeled with tomato lectin or 6H4 was measured.

Area measurements and neuronal counts were made using the ColorView IIu image capture and CellD image analysis system (Soft Imaging System; Olympus, Southall, UK). The level at which the hippocampus (and subiculum) was examined corresponded to the level at which the characteristic “U”-shaped configuration of the granule cells of the dentate gyrus was apparent (20). The hippocampus and subiculum were outlined under a 5× objective, and the area of the right and left hippocampus and subiculum was measured bilaterally in 2 consecutive sections, approximately 100 μm apart, and the means determined for each mouse. All labeled neurons that included the nucleus in the plane of section were counted. All neuronal counts were done under a 40× objective (captured image area, 0.07 mm²).

Data are presented as mean ± SEM. The data were analyzed by linear regression (GraphPad Prism, version 4.0 for Windows; Graphpad Software, San Diego, CA; www.graphpad.com). The analyses comprised i) simple linear regression, separately testing the null hypothesis for the NBH and ME7 groups that the slope of the regression line relating the number of neurons to the time after injection was zero and ii) comparison of the slopes of the regression lines for the 2 groups, testing the null hypothesis that the slopes were identical.

RESULTS

Histopathologic Changes in the Ventral Hippocampus

Examination of hematoxylin and eosin-stained sections did not show any pathologic changes in control brains at any of the time points examined. ME7-related pathologic findings were apparent at 16 weeks p.i. and was characterized by vacuolation and gliosis. The changes become more prominent at 20 weeks when there was widespread vacuolation within the hippocampal formation and entorhinal cortex. There was a progressive reduction in the thickness of the ventral CA1 pyramidal cell layer in ME7-affected mice during the course of the disease (p = 0.0015) (data not shown).

FIGURE 1. Areas of CA1 and subiculum, and total number of neurons, in standard sections through these regions. Sections were examined under a 40× objective in and neuronal counts quantified in an area of 0.07 mm². No significant change in hippocampal (A) or subicular (B) areas, or in the total number of neurons in CA1 (C) or the subiculum (D) was seen during the 20 weeks of the study period. The data are presented as the mean ± SEM for 6 mice in each group at each time point. The best-fit linear regression lines are also shown. Open circles, normal mouse brain homogenate-injected mice; closed circles, ME7-injected mice; p.i., postinjection.
shown). No statistically significant change was seen in control mice ($p = 0.68$). When the slopes of the 2 linear regressions were compared directly, they were found to be significantly different ($p = 0.007$; not shown). There was no significantly different change in hippocampal (control, $p = 0.57$; ME7, $p = 0.82$) or subicular (control, $p = 0.98$; ME7, $p = 0.87$) areas in control and ME7-injected mice during the course of the study. There were no statistically significant changes in the total number of neurons per unit area in ventral CA1 (control, $p = 0.09$; ME7, $p = 0.07$) or subiculum (control, $p = 0.99$; ME7, $p = 0.50$) during the 20-week course (Fig. 1).

**CNS Inflammation and Accumulation of PrPSc in the Ventral Hippocampus**

Tomato lectin (from *L. esculentum*; Vector Laboratories) is a glycoprotein that binds N-acetylglucosamine oligomers and is a marker of blood vessels and microglia in rodents. Examination of sections 4, 8, and 12 weeks p.i. revealed positive endothelial cell staining and scattered microglia with slender, elongated processes, and no qualitative difference between control and ME7-injected mice. At 16 weeks p.i., there were increased numbers of activated microglia with retracted shortened processes in the ventral hippocampal formation and, to a lesser extent, in the entorhinal cortex of ME7-injected mice (Fig. 2). By 20 weeks p.i., the numbers of activated microglia had increased further, and by this time, activated microglia were also evident in the entorhinal cortex (not shown). Sections from age-matched controls (at 16 and 20 weeks p.i.) contained relatively sparse, delicately ramified resting microglia and did not show morphologic evidence of microglial activation at either of these time points. Disease progression over time was characterized by increasing microglial activation. A signifi-

![FIGURE 2](image-url)

**FIGURE 2.** Microglial activation and accumulation of PrPSc at 16 weeks postinjection. Tomato lectin staining in the ventral hippocampus (CA1) of animals injected with normal mouse brain homogenate (NBH) (A) or ME7 (C) demonstrates the constitutive labeling of blood vessels in control and prion-affected tissue and increased numbers of activated microglia (arrows) in prion disease (C). Immunohistochemistry for PrPSc is negative in a section of CA1 from an animal injected with NBH (B). An ME7-affected brain (D) has a predominately synaptic pattern of PrPSc immunoreactivity, with only occasional plaque-like deposits. Scale bars = 50 μm.
FIGURE 3. Graphs illustrating the time course of microglial activation (A, B), PrP<sup>Sc</sup> accumulation (C, D), and loss of Wisteria floribunda agglutinin (WFA)-positive (E, F) and the number of parvalbumin (PV)-positive (G, H) neurons in the ventral CA1 (A, C, E, G) and subiculum (B, D, F, H) in ME7-affected mice compared with controls. The data are presented as the mean ± SEM for 6 mice in each group at each time point. The neuronal counts represent the average numbers of WFA- or PV-positive neurons in a unit area of ventral CA1 or subiculum derived from counts of 3 to 4 fields. Best-fit linear regression lines are indicated in (A, B, E, H); best-fit curves are in (C and D). Open circles indicate normal mouse brain homogenate-injected mice; closed circles, ME7-injected mice. (A, B) Microglial activation is increased in the CA1 and subiculum of ME7-injected mice but not in controls. (C, D) There is an exponential increase in PrP<sup>Sc</sup> accumulation in the CA1 and subiculum in ME7-affected brains but not controls. (E, F) There is progressive reduction in the numbers of WFA-positive neurons in both the CA1 and subiculum in ME7-injected mice but not controls. (G, H) There is no significant change in the number of PV-positive neurons in the CA1 and subiculum of control and ME7-injected mice during the course of the disease and no significant difference between the groups. p.i., postinjection.
cent direct linear relationship was demonstrable between the
duration of disease and the degree of microglial activation,
that is, proportions of the ventral CA1 and subiculum that
were tomato lectin positive (CA1, p = 0.03; subiculum, p = 0.05) (Fig. 3A, B). No significant increase in microglial
activation was seen in age-matched controls over the same
time course (CA1, p = 0.21; subiculum, p = 0.40). When the
slopes of the 2 regression lines were compared directly, they
were found to be significantly different (CA1, p = 0.01; ME7,
p = 0.03).

No 6H4-positive deposits were present in control mice
at any time point. At 8 weeks p.i., 2 of 6 ME7-injected mice
had occasional small 6H4-positive plaque-like deposits in
the hippocampus or subiculum. At 12 weeks p.i., there was a
variable amount of 6H4 immunopositivity ranging from
occasional plaque-like deposits (50% of cases) to multiple
deposits, most prominently in the dentate gyrus and CA2
hippocampus subfields (50% of cases). By 16 weeks p.i., the
changes were more consistent and characterized by PrPSc
deposits throughout the hippocampus (CA1, CA2, and CA3
subfields and dentate gyrus), subiculum, and entorhinal
cortex (Fig. 2). Both plaque-like deposits and finely granular
deposits, that is, a synaptic pattern of staining, were
observed. The distribution of 6H4 immunopositivity was
similar at 20 weeks p.i., with a more prominent synaptic
staining pattern.

WFA-Positive Neurons in the Ventral
Hippocampus

Wisteria floribunda agglutinin binds the N-acetylgalactosamine residues of CSPGs and has been reported to reveal
the PN most comprehensively in the mouse hippocampus. It
shows an overlapping pattern of staining with other antibody-
based reagents available for the demonstration of the PN (11).

The number and distribution of neurons with a PN
were similar in ME7-injected and control brains at 4 weeks
p.i.; no qualitative difference can be identified between the
groups at 8 weeks p.i. Wisteria floribunda agglutinin lectin
stained a number of nonpyramidal neurons scattered through-
out the hippocampus, subiculum, and entorhinal cortex. In
the hippocampus, the PN-positive neurons were located
either within the pyramidal cell layer or just outside it. Lectin
staining was present around individual neuronal cell bodies
and proximal dendrites. In addition, there was diffusely WA
lectin positivity within the neuropil of the CA1 pyramidal
cell layer, subiculum, and entorhinal cortex.

In ME7-infected mice, the numbers of WFA-positive
neurons per unit area of CA1 declined significantly during the
experimental course (p < 0.0001), whereas there was no such
decline in age-matched controls (p = 0.97; Fig. 3E). When the
slopes of the 2 linear regressions were compared, they were
found to be significantly different (p = 0.005). Similarly, in
the subiculum, ME7-injected mice had a progressive decline
in numbers of WFA-positive neurons (p = 0.0016) that was
not seen in age-matched controls (p = 0.70; Fig. 3F; Table 1).
When the 2 regression lines were compared, they were not
found to be significantly different (p = 0.06), which may be a
consequence of the small sample size.
At 12 weeks p.i., there was a qualitative reduction in the WFA labeling of neurons in 4 of the 6 ME7-injected mice examined. The reduction consisted predominantly of loss of peridendritic, rather than perisomatic staining and was associated with a reduction in the diffuse staining of neuropil within the CA1 subfield of the hippocampus, the subiculum, and entorhinal cortex. At 16 and 20 weeks p.i., there was a further reduction in the number of WFA-positive neurons (Table 1; Fig. 4A, B). The ME7-injected mice show an obvious reduction in WFA staining, particularly along dendrites. (C, D) Parvalbumin labeling is relatively preserved. (E, F) Double-fluorescent labeling of PV (red) and perineuronal net (PN) (green) in sections from ME7-injected mice. (E) At 8 weeks postinjection, PV-positive neurons (red) are surrounded by a PN (green) in a somatic and peridendritic distribution. (F) At 16 weeks, some PV-positive neurons lack a PN, and others show perisomatic but not peridendritic staining. Scale bar = 50 μm.

**PV-Positive Neurons in the Ventral Hippocampus**

No qualitative differences were observed in the distribution of PV labeling in control, and ME7 sections at 4, 8, and 12 weeks p.i. PV-positive neurons were seen throughout the CA1, CA2, and CA3 hippocampal subfields; subiculum; and in the entorhinal cortex. The antibody labeled the cell bodies of nonpyramidal neurons, and in some neurons, the labeling extended into the proximal dendrites. Within the hippocampus, PV-positive neurons were scattered within the pyramidal cell layers and dispersed outside it. In addition, there was prominent finely granular staining of the neuropil.

The numbers of PV-positive neurons per unit area of CA1 did not decline significantly during the experimental course either in ME7-injected mice (p = 0.25) or in age-matched controls (p = 0.16; Fig. 3G). Similarly, there were no significant reductions in the numbers of PV-positive neurons in the subiculum of ME7 (p = 0.09) or control mice (p = 0.10; Fig. 3G, Table 2).

There were no qualitative differences between control and ME7 mice in PV-positive neurons at 16 weeks p.i. (Fig. 4C, D), but there was PV-immunopositivity in the neuropil in 4 of 6 ME7 mice compared with 1 of 6 controls. Parvalbumin immunopositivity also seemed to be present in microglia-like cells in ME7-injected mice (not shown). There was also an increase in PV labeling within the neuropil, including within some microglia-like cells. Although there was some PV
immunopositivity in the neuropil in age-matched controls at this time (sections from 2 of 6 animals, included cells with microglia-like morphology), this was less abundant than in ME7-injected brains.

**WFA- and PV-Positive Neurons in the CA1 Region of the Ventral Hippocampus**

Double-fluorescent labeling of control sections showed that all PV-positive neurons were surrounded by a PN. In ME7-injected mice, the PN can be seen to surround PV-positive neurons at early stages of the disease (4 and 8 weeks), but from 12 weeks p.i., the sections included several PV-positive neurons that lacked a PN or had a PN that was largely restricted to the perikaryal region (Fig. 4E, F).

**DISCUSSION**

We have shown a progressive loss of PN from around neurons in the CA1 subfield of the ventral hippocampus and subiculum in the ME7 murine prion disease model. The earliest change in both CA1 and the subiculum seems to be PN loss in a peridendritic distribution, sparing the PN around individual neuronal cell bodies. Our data suggest that PN loss commences at approximately 12 weeks p.i.—approximately the same time as the limited accumulation of PrPSc (in 2/6 cases) and soon after microglial activation. Perineuronal net loss also seems to coincide with the onset of electrophysiological and behavioral abnormalities that we previously documented in this model (6).

Loss of the PN has been reported in human CJD, in terminal stages of experimental prion disease, and in the early stages of experimental murine CJD (16, 17). Guentchev et al. (16) demonstrated a loss of PV-positive interneurons from as early as one third of the way through the course of the experimental disease but observed that this occurred after the loss of the PN. Our results do not seem to show a significant loss of the PV-positive interneurons at early stages of the disease, although it would be premature to conclude that this is the case for later stages of the disease. The difference between our findings and those of Guentchev et al may reflect the use of different experimental models, that is, with different mouse scrapie strains, pathologic profiles, and incubation periods. Moreover, the PN- and PV-positive interneurons were quantitated in different brain regions, that is, in the neocortex in the study of Guentchev et al versus the ventral hippocampus in the present study. Our results are, however, consistent with those of Baig et al (21), who demonstrated the preservation of neocortical PV interneurons in Alzheimer disease in the context of significant neocortical PN loss. A more comparable study may be that of Brady and Mufson (22), who compared the number of PV-positive interneurons in the hippocampal formation of patients with Alzheimer disease with age-matched controls and found region-specific differences in the number of PV-positive interneurons. A significant reduction was reported in the dentate gyrus/CA4 and CA1-CA2 subfields, but no significant decline was present in the CA3 subfield, subiculum, or presubiculum. The preservation of PV-positive interneurons in the subiculum occurs despite the fact that the

**TABLE 2. PV-Positive CA1 and Subicular Neurons in Control and ME7-Infected Mice**

<table>
<thead>
<tr>
<th></th>
<th>Average no. neurons per field (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NBH ME7 NBH ME7 NBH ME7 NBH ME7 NBH ME7</td>
</tr>
<tr>
<td>Subiculum</td>
<td>109.18 ± 4.59 95.26 ± 0.85 74.83 ± 4.93 74.51 ± 6.09 96.5 ± 6.09</td>
</tr>
<tr>
<td>CA1</td>
<td>73.6 ± 4.24 68.88 ± 4.24 68.82 ± 1.36 72.07 ± 5.11 67.83 ± 4.24</td>
</tr>
<tr>
<td>Total</td>
<td>182.8 ± 9.23 164.14 ± 2.72 143.68 ± 6.32 146.58 ± 11.20 164.33 ± 10.33</td>
</tr>
</tbody>
</table>
| NBH normal mouse brain homogenate; p.i. = postinjection; PV, parvalbumin.
subiculum is a site of severe pathologic findings in Alzheimer disease. Although PV-positive interneurons were not lost from the regions examined in our study, the observation of PV immunopositivity in the neuropil and in microglia-like cells from 16 weeks onward raises the possibility that the cells or cell processes are damaged. The nature and pathogenesis of the possible damage to interneurons are the subjects of an ongoing study.

The reduction in the number of neurons with a PN occurred in the absence of significant changes in the area of the hippocampus or subiculum, or in the number of neurons per unit area of ventral CA1 or subiculum, up to 20 weeks p.i. This is noteworthy because previous studies have indicated that neurons were lost from the dorsal hippocampus in the CA1 subfield (3, 23), the loss commencing at 19 or 20 weeks p.i. and becoming most marked at 24 weeks (i.e. the terminal stage, a time point not included in the present study). Our findings suggest a difference in the neuronal vulnerability to injury in the dorsal and ventral hippocampus. Of possible relevance is the work of O’Donnell et al (24), who showed expression of the galanin receptor subunit GALR1 in the ventral but not in dorsal CA1 or subiculum. Galanin has been shown to have neuroprotective effects in the hippocampus, which are believed to be mediated via the GALR1 and GALR2 receptor subunits (25). Despite the preservation of neuronal numbers in the ventral CA1 in ME7-affected mice, and in keeping with our previous findings (3), we observed a significant reduction in the thickness of the CA1 pyramidal cell layer. This may reflect reduction in the surrounding ECM in this part of the hippocampus or in the size or dendritic arbor of individual neurons that were not assessed in this study.

As previously demonstrated, ME7 murine prion disease is a robust experimental model, with a reproducible incubation period and a well-characterized evolution of pathologic changes. It is therefore possible for us to relate our findings to our previous observations in this model and to those described by other authors using the same model. We and others have shown that this murine model is characterized by early and progressive CNS inflammation and behavioral abnormalities (3–5, 26). The paradigms used to demonstrate these behavioral abnormalities had a common neurobiologic basis and reflect the functioning of hippocampal neurons.

In the present study, we examined the ventral hippocampus because we had previously used this region in electrophysiologic studies and had demonstrated a reduction in synaptic plasticity from 12 weeks p.i. (6). Our findings suggest strongly that the pathologic substrate of the reduction in synaptic plasticity in early murine prion disease is the loss of the PN from around PV-positive interneurons. Previous studies have shown that the ECM, and in particular the PN, is essential for normal LTP (or synaptic plasticity). Application of chondroitinase avidin-biotin peroxidase complex (which digests the glycosaminoglycan side chains from CSPG) to hippocampal brain slices causes a reduction in LTP (27). Transgenic mice lacking specific PN or ECM components (e.g. brevican and tenascin) also show a reduction in LTP (14, 28). The electrophysiologic changes observed in these studies are identical to those we have demonstrated in slices of brain from prion-affected mice.

The mechanisms underlying PN degradation in prion disease are not clear. It is possible that extracellular accumulation of PrPSc may result in mechanical disruption of the PN. Baig et al showed a loss of PN from the frontal cortex in Alzheimer disease but did not find a significant difference in the amount of accumulated Aβ in the areas of cortex with and without WFA-positive PNs (21). It is possible that a soluble protein intermediate may be involved in the degradation of the PN. An alternative and possibly more likely explanation is that the degradation of the PN is a consequence of microglial activation. Degradation of the PN is a feature of human immunodeficiency virus-encephalitis (29), a condition that does not involve the accumulation of abnormal protein but is characterized by prominent microglial activation. Indeed, the degradation of the PN in human immunodeficiency virus-encephalitis has been attributed to the production of proteases by activated microglia (29). Microglial activation is an early and progressive feature of murine prion disease (3). This study provides evidence of a concomitant early and progressive loss of PN.

Questions remain as to the identity of the factor(s) that mediate the loss of PN. Activated microglia are known to secrete a number of proteases (30–32). Several observations suggest that the MMPs may be key mediators of PN degradation. Two members of this large group of ECM-degrading enzymes have been shown to have direct CSPG-degrading properties in the brain: MMP2 and MMP-24 (33, 34), and Muir et al (35) have shown that MMP-2 and MMP-3 can degrade most of the components of the PN. More recently, degradation of brevican by a family of metalloproteases called ADAMTS (i.e. a disintegrin and metalloprotease with a thrombospondin motif) has been demonstrated (36). Furthermore, ADAMTS-mediated brevican degradation colocalized with areas of synaptic loss in a kainic acid model of acute neuronal toxicity (37). The conspicuous activation of microglia in murine prion disease is in keeping with an important role for proteases in the pathogenesis of this disease. An increase in MMP-3 and MMP-12 mRNA was reported in the terminal stages in the model of murine prion disease used in the present study (38). More recently, elevated levels of MMP-2 and pro–MMP-9 were detected in the cerebrospinal fluid of patients with CJD (39). The direct contribution of MMPs to PN degradation and electrophysiologic abnormalities is currently being investigated.

In our study, PV-positive interneuron numbers were not significantly reduced during the 20-week study period, but surviving PV interneurons that lacked a PN can readily be demonstrated at 16 weeks p.i. We speculate that PN loss may impair interneuronal functioning. There is evidence that the PN contributes to the fast-spiking physiology of PV-positive interneurons (9, 10). A pharmacologic reduction of GABAergic inhibition (i.e. inhibition of PV-positive interneurons) in the rat caused behavioral changes (40) that are similar to those reported for prion disease, suggesting that there is analogous dysfunction of inhibitory interneurons in
the prion model. Although there is no doubt that the loss of pyramidal neurons in the dorsal hippocampus is a conspicuous feature of end-stage prion disease, we did not identify a significant reduction in numbers of CA1 or subicular neurons in the ventral hippocampus. This may be related to biologic differences between distinct neuroanatomic regions. Our electrophysiologic data do indicate, however, that neuronal functioning is deranged and, as noted in the previous sentences, this might be explained by the loss of the PN. Because the PN contributes to the normal functioning of interneurons, it is not surprising that its loss might contribute to interneuronal malfunctioning and abnormal modulation of cortical neuronal circuitry. Disruption of inhibitory transmission of PV-positive interneurons has been implicated in a number of neuropsychiatric diseases associated with cognitive impairment, including schizophrenia and epilepsy (41).

In summary, we identified a progressive loss PN that seems to be an early pathologic manifestation in ME7-induced murine prion disease and occurs at approximately the same time as PrPSc accumulation and soon after microglial activation. We suggest that microglial activation contributes to the disruption of the PN, and this may underlie the malfunctioning of neurons, which manifests electrophysiologically as a reduction in synaptic plasticity and abnormalities in hippocampus-dependent behaviors. The precise mediators of the destruction of PN remain to be elucidated, although there is some evidence that this may be mediated by MMPs. We believe that PN-deficient/PV-positive interneurons in the ventral hippocampus are functionally impaired, contributing to the changes in synaptic plasticity we have documented in this model. Further studies will be required to address this hypothesis.

REFERENCES

36. Mayer J, Hamel MG, Gottschal PE. Evidence for proteolytic cleavage of brevican by the ADAMTS in the dentate gyrus after excitotoxic lesion of the mouse entorhinal cortex. BMC Neurosci 2005;6:52
40. Bast Y, Zhang W-N, Feldon J. Hyperactivity, decreased startle reactivity, and disrupted prepulse inhibition following disinhibition of the rat ventral hippocampus by the GABAA receptor antagonist picrotoxin. Psychopharmacology (Berl) 2001;156:225–33