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The Neuropathological Phenotype in Transgenic Mice Expressing Different Prion Protein Constructs

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The neuropathological phenotype in transgenic mice expressing different prion protein constructs

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SUMMARY

Neuropathologic examination of transgenic (Tg) mice which express different prion protein (PrP) constructs is essential because spongiform (vacuolar) degeneration of neurons, the distribution of PrP^{Sc} and whether PrP amyloid plaques form are the phenotypes of prion diseases. In Tg models of experimental scrapie, it was found that all of the parameters that define prion isolates ('strains') can be manipulated by changing the structure of PrP. In those studies, further evidence that PrP^{Sc} causes scrapie neuropathology and determines scrapie incubation time was obtained. In addition, the distribution of PrP^{Sc} in the brain was unique for each prion isolate. The implications of these findings are first, that prion isolates target different neuron populations for synthesis of nascent pathogenic PrP^{Sc} and, secondly, that prion isolate diversity is determined by neurons. In Tg mice which express mutated PrP mimicking human prion protein gene mutations linked to familial prion diseases, the neuropathological changes have been faithfully reproduced. A new age-related, neuromascular disorder has also been identified in uninfected Tg mice which overexpress wild-type PrP^{C}. All of the findings with different PrP constructs plus the absence of scrapie pathology in PrP null mice are the strongest argument that the prion protein is the main etiologic and pathogenic factor of prion disorders.

1. INTRODUCTION

A now extensive mass of varied experimental data consistently argue that the etiology and pathogenesis of all forms of Creutzfeldt–Jakob disease (CJD) and related human neurodegenerative disorders as well as scrapie in animals are associated with abnormalities of the prion protein (PrP) synthesized largely by CNS neurons (Prusiner 1991; DeArmond & Prusiner 1993). These disorders are similar to Alzheimer's disease because they include sporadic and familial cases; however, they are unique among primary neurodegenerative disorders because abnormal PrP forms into a particle, a prion, which can transmit them to other subjects. The evidence that the prion is composed mostly if not exclusively of abnormal PrP derives from studies which indicate that the scrapie agent cannot be inactivated by procedures which denature nucleic acids or inactivate viruses (Alper *et al.* 1967, 1978; Prusiner 1982; McKinley *et al.* 1983; Bellinger-Kawahara *et al.* 1987*a*,*b*), by chemical analysis of highly purified preparations of the scrapie agent which have shown that infectivity co-purifies with abnormal protease resistant PrP, PrP^{Sc} (Prusiner *et al.* 1982, 1983; Gabizon *et al.* 1987, 1988), and by sensitive physical–chemical methods which have shown that highly purified scrapie prion preparations do not contain viral nucleic acid (Meyer *et al.* 1991; Kellings *et al.* 1992). Recent studies with transgenic (Tg) mice have provided the most convincing corroborative evidence for the central role of PrP. These have verified that human genetic forms of these diseases are related to mutations of the PrP gene (designated PRNP in humans and *Prn-p* in animals) (See Hsiao & Prusiner 1990; Prusiner 1991; DeArmond & Prusiner 1993); that 'infectious' prions form spontaneously in Tg mice expressing pathogenic mutated PrP (Hsiao *et al.* 1994); that the host species barrier and different clinical–neuropathological subtypes of scrapie are determined by the structure of PrP (Scott *et al.* 1989; Prusiner *et al.* 1990); and that the parameters which define different scrapie prion isolates ('strains'), including scrapie incubation time, the distribution of spongiform degeneration and whether or not PrP amyloid plaques appear, can be manipulated by changing the amino acid sequence of PrP (Scott *et al.* 1993). The requirement of PrP in these disorders has been verified in PrP knockout mice. These $Prn\text{-}p^{o/o}$ mice do not develop clinical signs, neuropathology or form prions after inoculation with scrapie prions (Büeler *et al.* 1992); however, when the *Prn-p* gene is added back to the null mice, $Prn\text{-}p^{+/o}$ mice, the clinical, neuropathological and infectious characteristics induced by inoculation of prions return (Büeler *et al.* 1993; Prusiner *et al.* 1993).

The discovery of the prion protein in highly purified

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preparations of the scrapie agent, the pioneering molecular genetic studies of familial human prion diseases and the development and refinement of PrP transgenic mouse models have made it possible for the first time to examine the molecular and cellular mechanisms of PrP disorders. Although the preeminence of PrP has been established by these studies, many more questions have been raised regarding how an infectious agent composed solely of an abnormal variant of a single brain protein can transmit disease and code for a variety of clinical–neuropathological syndromes. Equally challenging is explaining sporadic cases of CJD which account for 85% of the total number of human prion diseases.

Here, we will review some of our recent findings from Tg mice expressing different PrP transgenes from the perspective of neuropathology and neuroanatomy. Although this may seem an unlikely source for new understanding of the etiology and pathogenesis of sporadic, infectious and genetic prion diseases, it has been very fruitful because the most pertinent phenotypes of prion diseases are the distribution and intensity of spongiform degeneration, PrP amyloid plaque formation and the distribution of abnormal PrP.

2. THE SCRAPIE PRION PHENOTYPE: DISTRIBUTION OF PrP^{Sc} IS THE MOST FUNDAMENTAL PARAMETER

(*a*) *The scrapie incubation time gene and* Prn-p *are tightly linked*

That a single host animal species can synthesize multiple kinds of prions was first discovered during laboratory transmission of scrapie among sheep strains and goats (Pattison & Millson 1961). Differences among prion isolates could be distinguished by clinical signs and scrapie incubation time. More than 15 different scrapie prion isolates were subsequently obtained from rodents (Bruce & Fraser 1991), which were distinguished by scrapie incubation time, the distribution and intensity of spongiform degeneration in the nervous system and whether or not cerebral amyloid plaques formed (Fraser & Bruce 1973; Fraser & Dickinson 1973; Bruce *et al.* 1976). These characteristics are faithfully reproduced during multiple sequential transmissions of a given prion isolate in a single mouse or hamster strain but vary markedly or even fail to appear when transferred to a different animal species (host species barrier). Although scrapie incubation time is determined in part by the infecting prion, classic genetic studies in different mouse strains have indicated that a single autosomal gene also influences scrapie incubation time (Dickinson & Meikle 1971). After the discovery of PrP, molecular genetic studies showed that the scrapie incubation time gene is tightly linked to or identical with the *Prn-p* gene (Carlson *et al.* 1986).

(*b*) *PrP^{Sc} accumulation in the brain causes the clinically relevant neuropathology*

The other differentiating characteristics of prion isolates, the distribution and intensity of spongiform degeneration and formation of amyloid plaques, have also been found to be causally linked to PrP. Amyloid plaques in prion disorders contain protease resistant PrP (DeArmond *et al.* 1985, 1987; Prusiner & DeArmond 1987; Roberts *et al.* 1988; Snow *et al.* 1989). Localization of PrP^{Sc} in the brain by neurochemical methods, by immunohistochemistry and by histoblot analysis have revealed a precise topographical correlation between the sites of PrP^{Sc} accumulation, spongiform degeneration and reactive astrocytic gliosis. This relationship has been found with the Sc237 and 139H isolates in Syrian hamsters (DeArmond *et al.* 1987; Hecker *et al.* 1992); the Sc237, 139H and Me7H isolates in Tg mice expressing Syrian hamster (SHa) PrP (Hecker *et al.* 1992; DeArmond *et al.* 1993); and both the Sc237 and RML isolates in Tg mice expressing a chimeric PrP containing both mouse (Mo) and SHa PrP sequences (figure 1) (Scott *et al.* 1993). In addition to this spatial relationship, there is also a temporal correlation between the accumulation of PrP^{Sc} in a brain region and the development of neuropathology (Jendroska *et al.* 1991). However, the most convincing arguments that abnormal PrP causes pathology are the molecular genetic studies of familial prion diseases which have linked mutations in the PRNP gene with development of brain pathology and clinical signs. The pathogenic effect of the PRNP codon 102 mutation found in families with the ataxic form of Gerstmann–Sträussler–Scheinker (GSS) syndrome (Hsiao *et al.* 1989*a*,*b*), has been verified in Tg mice expressing a *Prn-p* transgene mimicking the human mutation (Hsiao *et al.* 1990). These Tg(GSSMoPrP101L) mice spontaneously develop a neurological disorder characterized by spongiform degeneration of grey matter, formation of kuru-type PrP amyloid plaques in multiple brain regions (figure 2), and formation of prions (Hsiao *et al.* 1994).

(*b*) *The rate of accumulation of PrP^{Sc} determines scrapie incubation time*

The data reviewed above imply that the rates of formation and accumulation of PrP^{Sc} determine the rate of formation of clinically relevant neuropathology and, therefore, influence scrapie incubation time. In this regard, scrapie incubation time correlates well the amount of SHaPrP mRNA expressed in different Tg mouse lines expressing SHaPrP (Prusiner *et al.* 1990). In different hamster species inoculated with the Sc237 prion isolate (DeArmond *et al.* 1992), and in Syrian hamsters inoculated with either the Sc237 or 139H isolates (Hecker *et al.* 1992), incubation time was also found to be a function of the rate and pattern of PrP^{Sc} accumulation.

(*d*) *Prion isolates target specific neuron populations for synthesis of PrP^{Sc}*

The high sensitivity of the histoblot technique for localizing and quantifying PrP^{Sc} (Taraboulos *et al.* 1992) has revealed that the neuroanatomic location of PrP^{Sc} accumulation is unique for each scrapie prion isolate and not a function of scrapie incubation time

(Hecker *et al.* 1992; DeArmond & Prusiner 1993; DeArmond *et al.* 1993). In comparing the kinetics of PrP^{Sc} accumulation in the brain of Syrian hamsters inoculated with either Sc237 or 139H prions, we found that its distribution at the time clinical signs became apparent was markedly different. Specifically, PrP^{Sc} was localized to fewer brain regions with Sc237 than with 139H; however, because scrapie incubation time was significant longer with 139H, 170 days versus 70 days for Sc237, we could not rule out the possibility that the longer incubation time with 139H permitted the disease to spread non-specifically to more brain regions rather than showing selective targeting. The influence of scrapie incubation time was eliminated in studies with Tg(SHaPrP)-7 mice which express high levels of SHaPrP mRNA because scrapie incubations times were virtually identical for the Sc237 and 139H isolates, approximately 50 days (Hecker *et al.* 1992). In these as well as all other studies described here, prions were inoculated unilaterally into the thalamus. With the Sc237 isolate, PrP^{Sc} accumulation was largely confined to selected nuclei in the thalamus, septum and brainstem while it was widely distributed throughout the CNS with 139H (figure 3). With the Me7H isolate, PrP^{Sc} accumulation was even more restricted being confined to the paraventricular nucleus of the thalamus, the habenula, the hypothalamus, zona incerta, nucleus accumbens septi, and periaqueductal grey of the midbrain (DeArmond *et al.* 1993). These findings were remarkable because scrapie incubation time with Me7H in Tg(SHaPrP)-7 mice was about 185 days and, therefore, the highly restricted distribution of PrP^{Sc} could not be attributed to insufficient time for prions to spread in the CNS.

Linkage of the scrapie incubation time gene with the *Prn-p* gene, the correlation between the rate and pattern of PrP^{Sc} accumulation in the brain and scrapie incubation time, the causal relationship between PrP^{Sc} accumulation and the clinically relevant neuropathology, and selective targeting of different brain regions by each prion isolate independent of scrapie incubation time has two implications. First, they argue that the neuroanatomical pattern of PrP^{Sc} accumulation in the brain is the most fundamental parameter which identifies and differentiates scrapie prion isolates. Secondly, they suggest a mechanism for the origin of multiple prion isolates from a single animal within the constraints of the hypothesis that the sole functional component of prions is PrP^{Sc}.

3. PrP^{Sc} STRUCTURE AND SCRAPIE PRION ISOLATES: THE NEURON ORIGIN HYPOTHESIS

The possibility that the sole functional component of prions is PrP^{Sc} implies that information for the strain-like behaviour of prions is encoded in its structure. The fact that a single animal can form multiple stable prion isolates each causing different clinical–neuropathological diseases suggests that multiple stable structural isoforms of PrP^{Sc} are possible. Because nascent PrP^{Sc} is derived from the normal cellular isoform, PrP^{C}, as the result of a post-translational

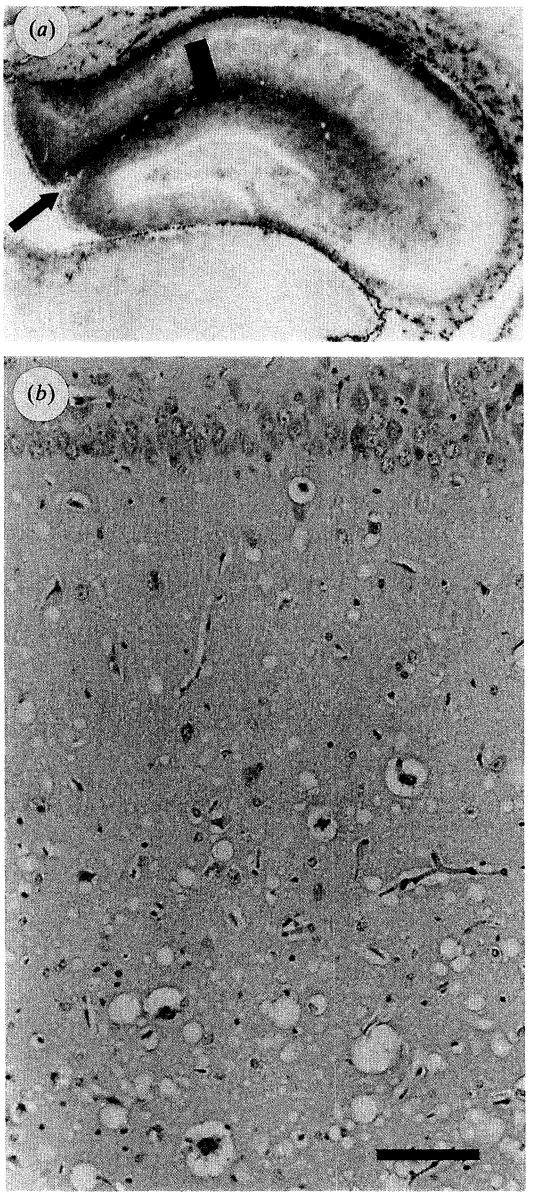

Figure 1. Spongiform degeneration of grey matter colocalizes with PrP^{Sc}. Represented is Syrian hamster brain inoculated with the RML prion isolate passaged once in a Tg(MH2M PrP) mouse which expressed a chimeric PrP^{C} composed in part of mouse and in part of Syrian hamster PrP sequences. (*a*) Histoblot of hippocampus show that the most intense PrP^{Sc} signal occurs in the region of the hippocampal fissure (arrow). The black rectangle indicates the approximate location of the photomicrograph in (*b*). (*b*) Haematoxylin and eosin stained section of the hippocampus show that the most intense spongiform degeneration occurred where the PrP^{Sc} signal was most intense. Bar in (*b*) is 50 μm.

mechanism which appears to require an interaction between pre-existing PrP^{Sc} and PrP^{C} (PrP^{Sc}–PrP^{C} dimer hypothesis) (Borchelt *et al.* 1990, 1992; Caughey *et al.* 1991), there are two possibilities for diversity of PrP^{Sc} structure. First, each cell may synthesize a single PrP^{C} isoform with the same amino acid sequence and same secondary structures. In this case, PrP^{Sc} would have to transfer multiple stable structural configurations to PrP^{C}. Alternatively, each cell type may synthesize a different isoform of PrP^{C} with the same

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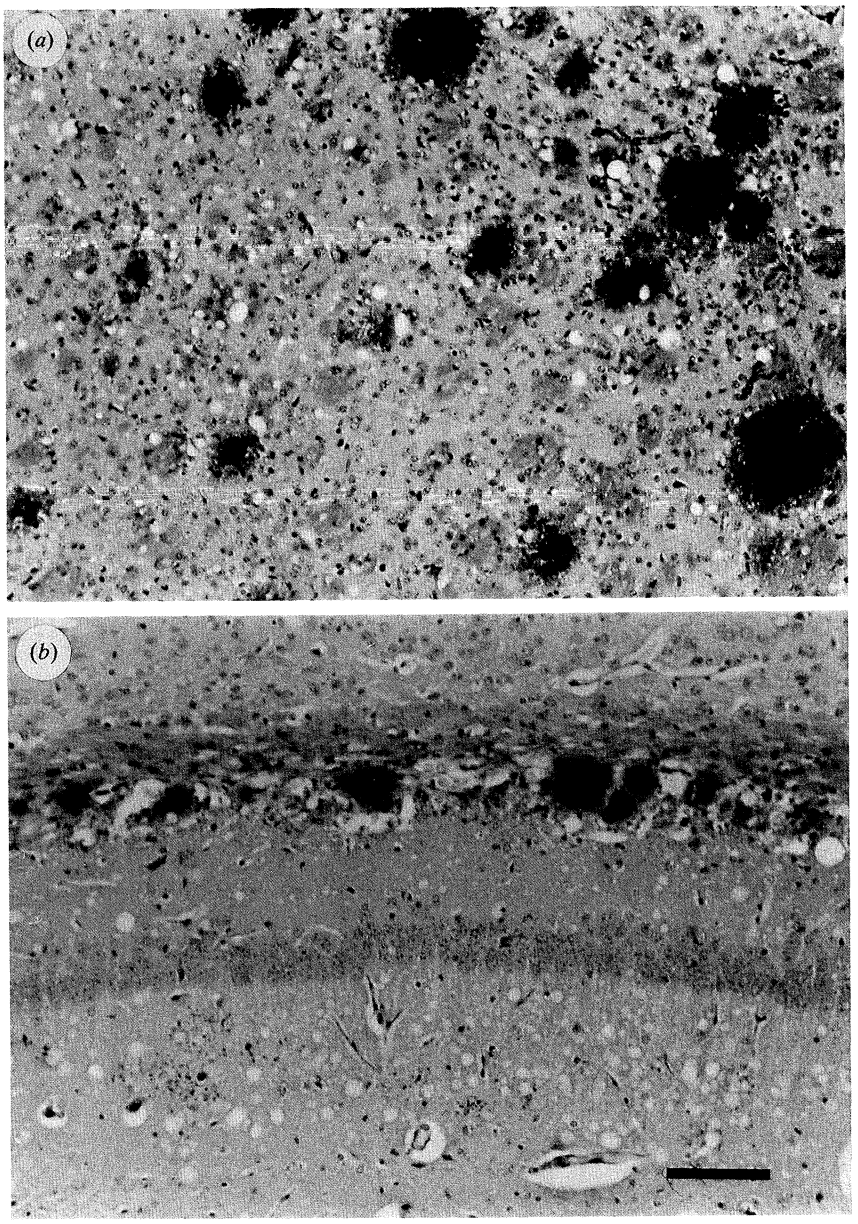

Figure 2. Amyloid plaques develop in Tg(MoPrP-P101L) mice which express PrP with a mutation which mimics the PRNP codon 102 mutation linked to Gerstmann–Sträussler syndrome. (*a*) In the Tg-174H line which develops neurodegeneration spontaneously, multiple amyloid plaques are located in the caudate nucleus. (*b*) In the Tg-196L line, neither neurodegeneration nor amyloid plaque formation occurred spontaneously; rather, neurodegeneration required inoculation with prions, in this case, prions in a brain homogenate derived from Tg-174H mice. Amyloid plaques were found exclusively beneath the corpus callosum overlying the hippocampus. Periodic acid-Schiff stain. Bar is 50 μm. (Adapted from Hsiao *et al.* 1994.)

amino acid sequence but different secondary, tertiary or quaternary structures. For example, it is possible that each neuron synthesizes PrP^{C} molecules with different carbohydrate trees. In this case, the structural differences in PrP^{Sc} molecules which hypothetically underlie scrapie isolate diversity would be determined by existing structural differences in PrP^{C} from which PrP^{Sc} is derived. There are several reasons to believe that the second possibility is correct.

First, the interaction between PrP^{Sc} of the infecting prion and PrP^{C} is specific and selective. This was first recognized in Tg(SHaPrP) mice which express both $SHaPrP^{C}$ and $MoPrP^{C}$ (Scott *et al.* 1989; Prusiner *et al.* 1990). When these animals were inoculated with SHa-adapted Sc237 prions, only $SHaPrP^{Sc}$ was formed based on the characteristics of the neuropathology, the presence of $SHaPrP^{Sc}$ in the neuropil and in amyloid plaques, and the behaviour of the newly formed prions which had characteristics of Sc237 prions. In contrast, Mo-adapted RML prions only interacted $MoPrP^{C}$ since neuropathology characteristic of RML scrapie developed. Therefore, Sc237 prions selectively interacted with $SHaPrP^{C}$ in these Tg mice and RML prions selectively interacted with $MoPrP^{C}$. Related to this homophilic interaction between PrP^{Sc} and PrP^{C}, the species barrier of mice to SHa-derived Sc237 prions had also been breached. The homophilic interaction of PrP^{Sc} with PrP^{C} was

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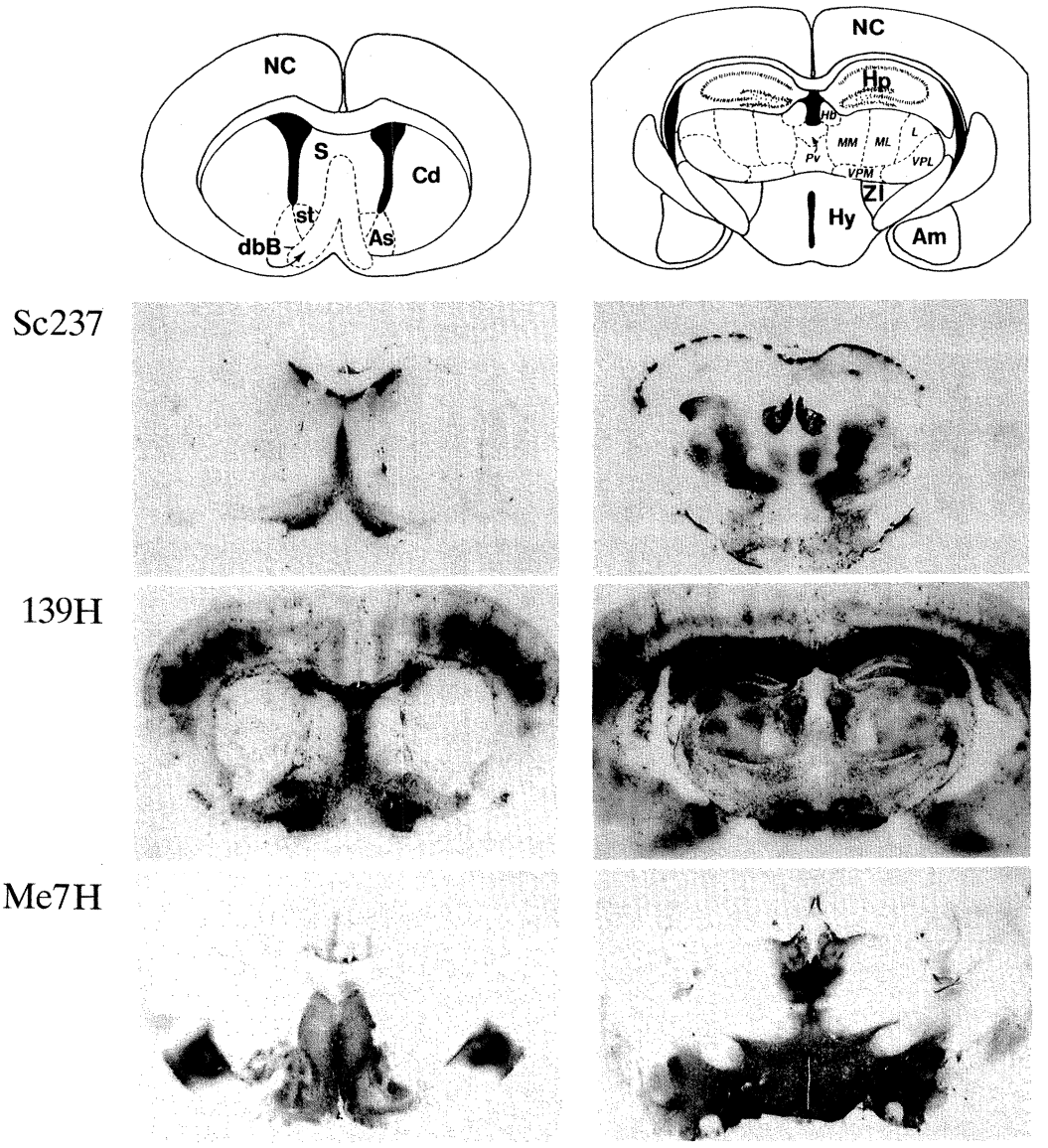

Figure 3. The regional distribution of PrP^{Sc} in the brain revealed by histoblots is unique for each prion isolate. In this study, Tg(SHaPrP)-7 mice, which express high levels of $SHaPrP^{C}$, were inoculated in the thalamus with either Sc237, 139H or Me7H prions. Two levels of the brain are shown. Mice inoculated with Sc237 or 139H prions develop clinical signs about 50 days after inoculation. For the Me7H isolate, animals develop clinical signs between 180 and 200 days. Am, amygdala; As, accumbens septi; Cd, caudate nucleus; dbB, diagonal band of Broca; Hp, hippocampus; Hy, hypothalamus; NC, neocortex; S, Septal nuclei; st, interstitial nucleus of the stria terminalis; ZI, zona incerta. Thalamic nuclei in italics: *Hb*, habenula; *L*, lateral; *ML*, medial, pars lateralis; *MM*, medial, pars medialis; *Pv*, paraventricular; *VPL*, ventral posterior lateral; *VPM*, ventral posterior medial.

more recently demonstrated in Tg mice expressing chimeric PrP^{C} containing amino acid sequences from both SHaPrP and MoPrP (Scott *et al.* 1993). Two chimeric constructs were made on the MoPrP background: one containing two amino acid substitutions from SHa at codons 108 and 111 designated MHM2 PrP and the other containing three additional substitutions at codons 138, 154 and 169 designated MH2M. Three Tg mouse lines expressing the former construct, Tg(MHM2) mice, were resistant to Sc237 derived from Syrian hamsters, SHa(Sc237), similar to non-Tg mice; however, all Tg mice expressing the transgene with five SHa amino acid substitutions, Tg(MH2M) mice, became clinically ill with SHa(Sc237) prions. This argues that the homophilic interaction between PrP^{Sc} in the prion and PrP^{C} in the host leading to a breech of the species barrier and clinical disease is related to the amino acid sequence of both molecules. When the resulting prions, designated MH2M(Sc237), were passaged back into Syrian hamsters, they developed scrapie characterized by distribution of PrP^{Sc} similar to, although not identical with, that caused by SHa(237). Mouse-derived RML prions, Mo(RML), also produced scrapie in the Tg(MH2M) mice. Although Mo(RML) prions do not infect Syrian hamsters, inoculation of brain homogenates from Tg(MH2M) mice into Syrian hamsters did infect 24 out of 24 animals indicating that MH2M(RML) prions had been formed. Furthermore, the pattern of PrP^{Sc} accumulation in Syrian hamster brain was unique indicating that the artificial prion created by the chimeric transgene was a new

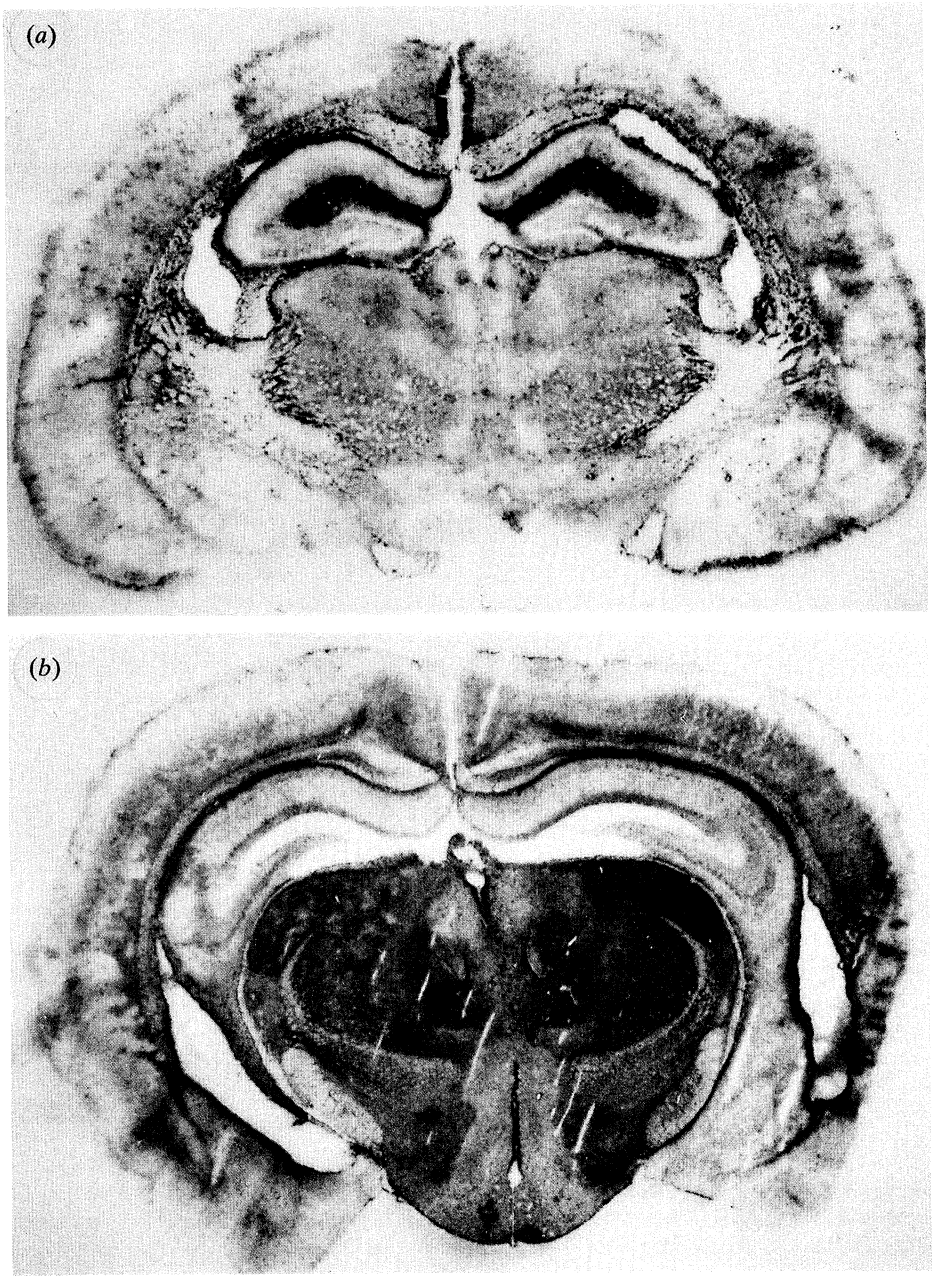

Figure 4. An artificial prion created in transgenic mice expressing a chimeric PrP^{C} produces a unique histoblot pattern of PrP^{Sc} accumulation in Syrian hamster. (*a*) Syrian hamsters were inoculated with a brain homogenate from Tg(MH2M) mice infected with RML prions. The most intense PrP^{Sc} signal occurs along the hippocampal fissure in the hippocampus (also see figure 1) and subpially at the periphery of the cerebral hemispheres and thalamus. The pattern is significantly different than that produced by Sc237 prions passaged among Syrian hamsters (*b*).

scrapie prion isolate (figure 4). Particularly unique aspects of the PrP^{Sc} pattern was its dense accumulation along the hippocampal fissure and subpially at the periphery of the brain. Both the MH2M(RML) and MH2M(Sc237) prions also transmitted to CD-1 mice, indicating that the amino acid sequence of this chimeric prion was able to overcome the natural species barrier of SHa(Sc237) in mice. These studies support the view that the interaction between the prion and PrP^{C} is specific and is consistent with the dimer hypothesis that PrP^{Sc} of the prion and the PrP^{C} in the host must interact to transform the latter into nascent PrP^{Sc}.

The selectivity of the PrP^{Sc} and PrP^{C} interaction required for nascent PrP^{Sc} formation coupled with the finding that each prion isolate targets a different population of neurons suggest that each neuron population synthesizes a different PrP^{C} isoform. Prion-stimulated conversion of PrP^{C} into nascent PrP^{Sc} would occur only in those neurons which synthesize an isoform of PrP^{C} compatible with the PrP^{Sc} of the infecting prion. This testable hypothesis not only provides a plausible explanation for the origin of multiple prion isolates from a single animal species but also provides a molecular explanation for the different clinical–neuropathological syndromes of scrapie.

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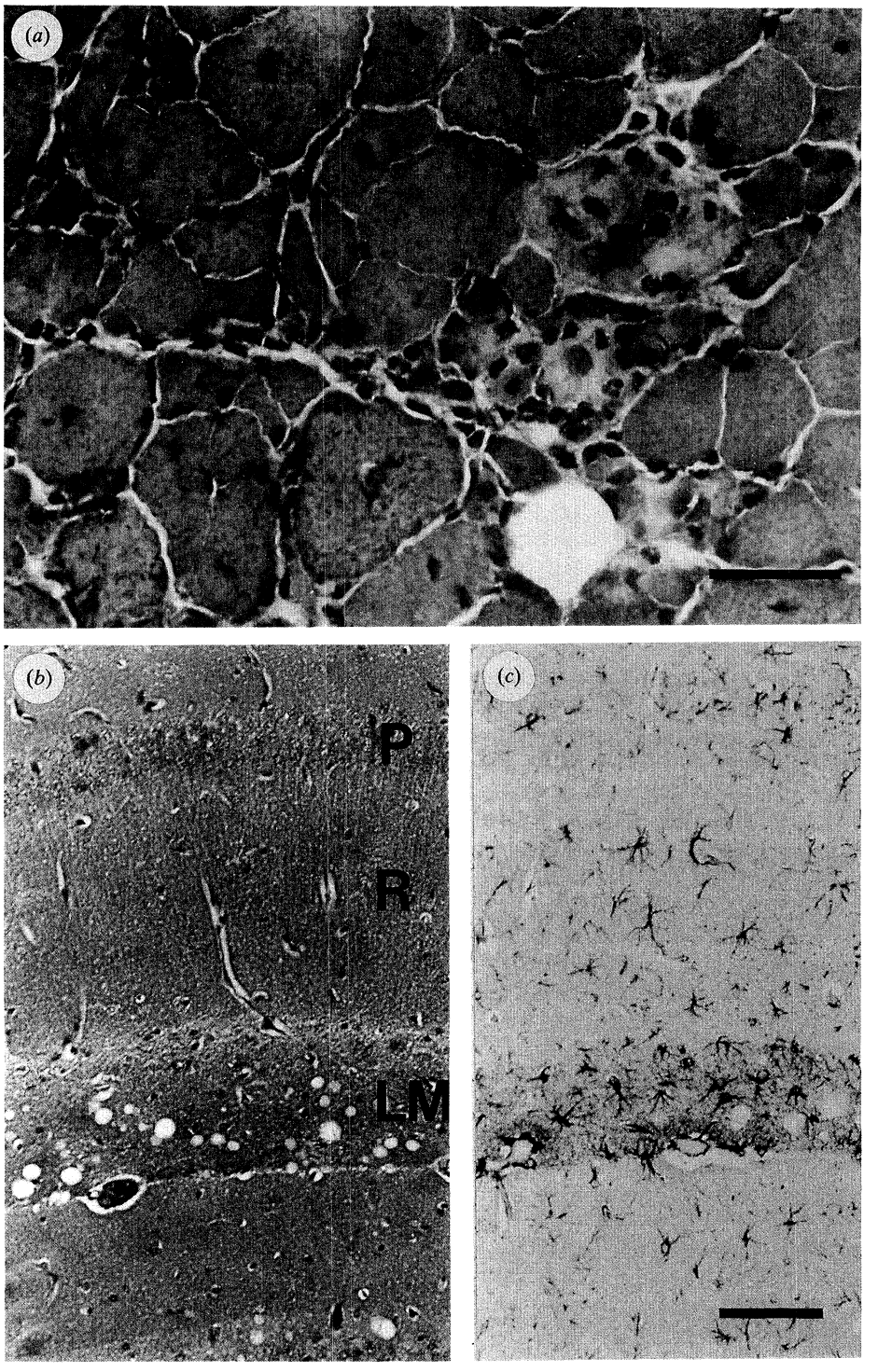

Figure 5. Clinical signs in uninfected transgenic mice which express high levels of wild-type PrP^C are due to a necrotizing myopathy (*a*) characterized by degeneration and phagocytosis of skeletal muscle. Vacuolar degeneration (*b*) and reactive astrocytic gliosis (*c*) in the CNS are confined to the stratum lacunosum moleculare (LM) of the hippocampus and are insufficient to account for clinical signs. P, pyramidal cell layer; R, stratum radiatum. Bar in (*a*) is 50 μm. Bar in (*c*) is 50 μm and also applies to (*b*). Haematoxylin and eosin stain (*a* & *b*), glial fibrillary acidic immunohistochemistry (*c*).

4. AGE-RELATED MYOPATHY, DEMYELINATING NEUROPATHY AND FOCAL VACUOLATION OF THE CNS IN MICE OVEREXPRESSING wtPrP^C

We recently discovered that a large proportion of old (400–550 day), uninfected Tg mice which express high levels of hamster, mouse or sheep wild-type (wt) PrP^C transgenes develop severe generalized skeletal muscle weakness (Westaway *et al.* 1994). In the CNS, the main neuropathological feature was mild vacuolization of the lacunosum molecular layer of the hippocampus which was insufficient to account for the clinical signs (figure 5*b*,*c*). The most intense pathology which best correlated with clinical signs was localized to skeletal muscle but not cardiac or smooth muscle.

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The myopathy is characterized by degenerating muscle fibres with some undergoing phagocytosis, central nuclei in most muscle fibres, marked variation in fibre size, endomysial fibrosis and fatty infiltration (figure 5*a*). No protease-resistant PrP was found in skeletal muscle; however, there were increased amounts of protease-sensitive PrP and, in the case of Tg7 mice which overexpress SHaPrP, the increase was due exclusively to SHaPrP^{C} accumulation. Necrotic muscle fibres could be identified as early as 90 days of age. These animals also developed a neuropathy dominated by demyelination. Neurogenic rearrangement of muscle fibres was associated with the latter; however, the clinical signs appeared to be directly related to the myopathy based on its severity and its early onset.

5. SPORADIC CJD

Preliminary transmission data suggests that Tg mice overexpressing wtPrP^{C} form infectious prions (see Prusiner, this symposium). If this last observation can be verified, it would provide one possible mechanism for the origin of sporadic CJD. Sporadic CJD accounts for at least 85% of human prion diseases. It is an age-related disorder with a peak onset at about 60 years of age. There are now three possible mechanisms to explain its occurrence. First, the fact that there are multiple mutations of the PRNP gene which are pathogenic raises the possibility of an age-related acquired mutation of the PRNP gene. If such a mutation led to the formation of pathogenic PrP in even a single neuron, the experience from infectious scrapie argues that a chain reaction could result which leads to the spread of disease to other susceptible neurons. Secondly, the remarkable pathology and prion formation found in Tg mice overexpressing wtPrP^{C} suggests an equally plausible hypothesis that sporadic CJD results from spontaneous conversion of PrP^{C} into pathogenic PrP in one or more subpopulations of cells. A third mechanism may be a genetic predisposition to environmental prions or to spontaneous conversion of PrP^{C} to PrP^{CJD} based on homozygosity at the normal PRNP polymorphism at codon 129 (Palmer *et al.* 1991; Brown *et al.* 1992; Collinge *et al.* 1992).

6. CONCLUDING REMARKS

The species barrier to scrapie, scrapie incubation time and the distribution and the characteristics of scrapie neuropathogy have been the parameters used to define scrapie prion isolates ('strains'). Our studies suggest that the distribution and amount of PrP^{Sc} among brain regions are the more fundamental phenotypic parameters which differentiate scrapie prion isolates because they are independent of scrapie incubation time and because PrP^{Sc} accumulation appears to cause the neuropathological changes characteristic of scrapie. Furthermore, several studies with transgenic mice expressing SHa and chimeric PrPs indicate that the scrapie species barrier is directly related to the PrP amino acid sequence. Thus our investigations of the etiology and pathogenesis from the perspective of neuroanatomy and neuropathology have consistently corroborated the hypothesis that PrP^{Sc} plays the preeminent role in both. These morphological studies have also generated a new hypothesis to explain the origin of different prion isolates in the context of the possibility that the sole functional component of prions is PrP^{Sc}. This new hypothesis suggests that the diversity of PrP^{Sc} structure required for prion diversity results from natural structural diversity of PrP^{C} determined by each cell population. The neuropathological investigations have verified that transgenic mice expressing mutated PrPs which mimic genetic forms of human prion diseases develop a clinical–pathological syndrome virtually identical to that which occurs in humans including spontaneous formation of prions. The precision with which these animal models reproduce genetic human prion diseases is the strongest arguement that the prion protein is the main etiologic and pathogenic factor in these disorders. The resistance of *Prn-po/o* null mice to scrapie infection is strong indirect evidence in support of the pre-eminence of the prion protein. Finally, the close correlation of neuropathological with molecular studies have shown that prion disorders need not arise solely from exposure of an animal to protease-resistant PrP^{Sc} in the form of prions or from endogenously synthesized mutated forms of PrP. Overexpression of wild-type PrP^{C} itself appears to be pathogenic and capable of leading to formation of prions spontaneously.

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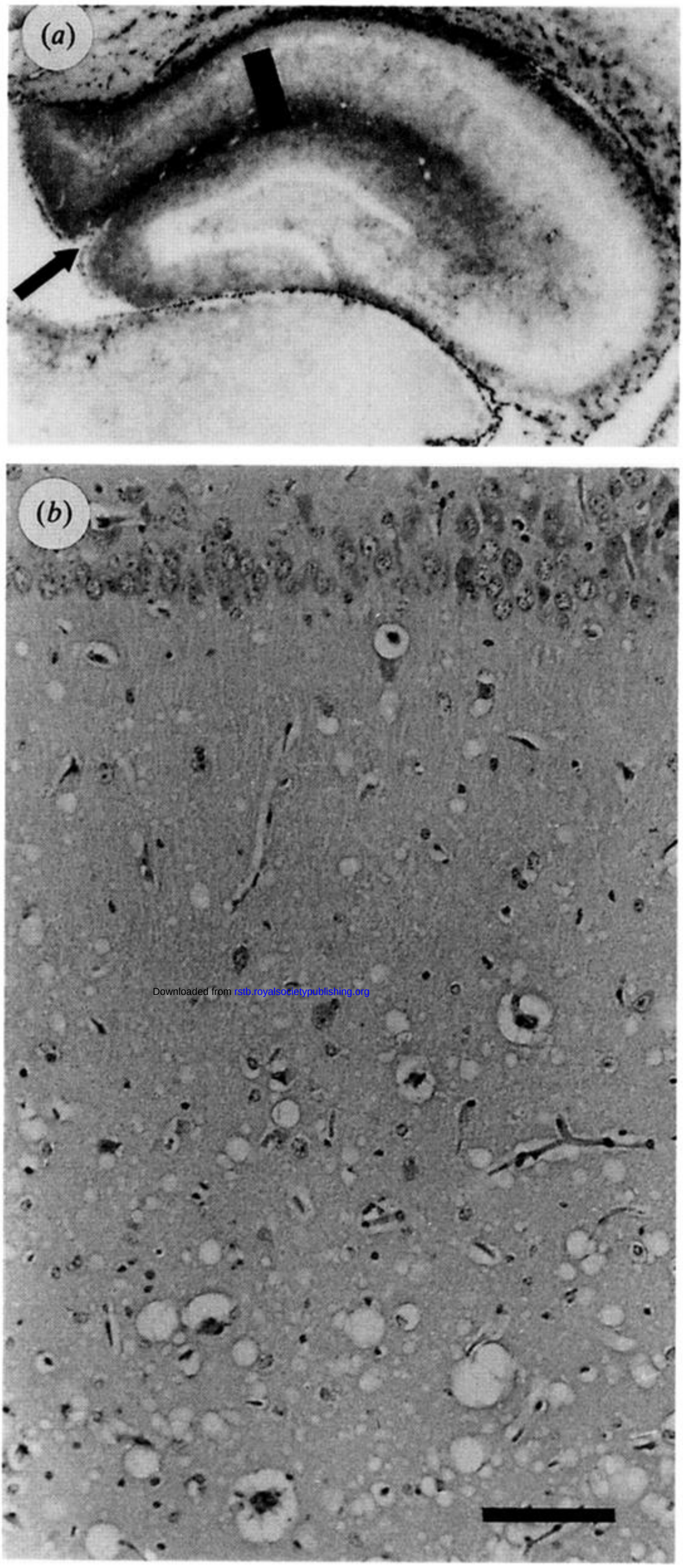

gure 1. Spongiform degeneration of grey matter coloca-
es with PrP^{Sc}. Represented is Syrian hamster brain
oculated with the RML prion isolate passaged once in a
g(MH2M PrP) mouse which expressed a chimeric PrP^{C}
mposed in part of mouse and in part of Syrian hamster
P sequences. (*a*) Histoblot of hippocampus show that the
ost intense PrP^{Sc} signal occurs in the region of the
ppocampal fissure (arrow). The black rectangle indicates
e approximate location of the photomicrograph in (*b*). (*b*)
aematoxylin and eosin stained section of the hippocampus
ow that the most intense spongiform degeneration
curred where the PrP^{Sc} signal was most intense. Bar in (*b*)
50 μm.

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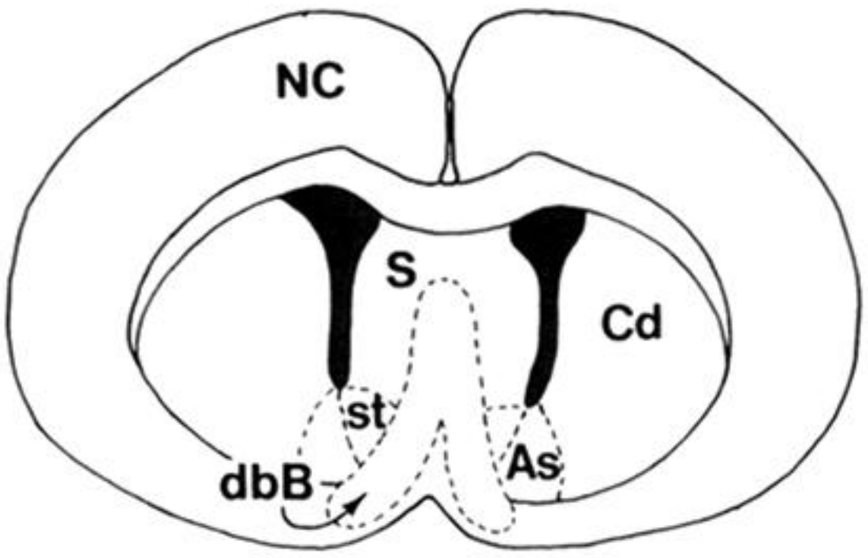
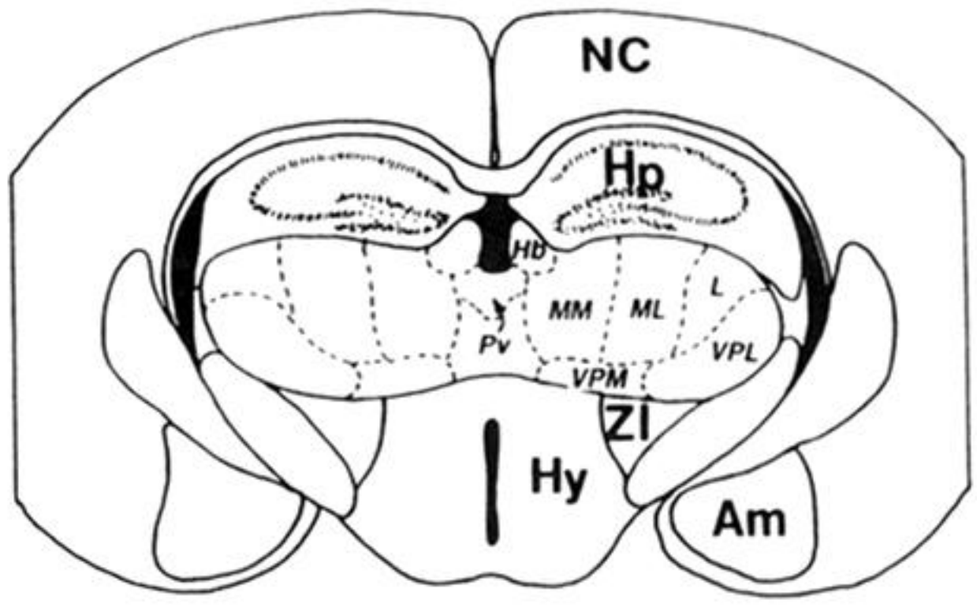
gure 2. Amyloid plaques develop in Tg(MoPrP-P101L) mice which express PrP with a mutation which mimics e PRNP codon 102 mutation linked to Gerstmann–Sträussler syndrome. (*a*) In the Tg-174H line which develops urodegeneration spontaneously, multiple amyloid plaques are located in the caudate nucleus. (*b*) In the Tg-196L ne, neither neurodegeneration nor amyloid plaque formation occurred spontaneously; rather, neurodegeneration quired inoculation with prions, in this case, prions in a brain homogenate derived from Tg-174H mice. Amyloid aques were found exclusively beneath the corpus callosum overlying the hippocampus. Periodic acid-Schiff stain. ar is 50 μm. (Adapted from Hsiao *et al.* 1994.)





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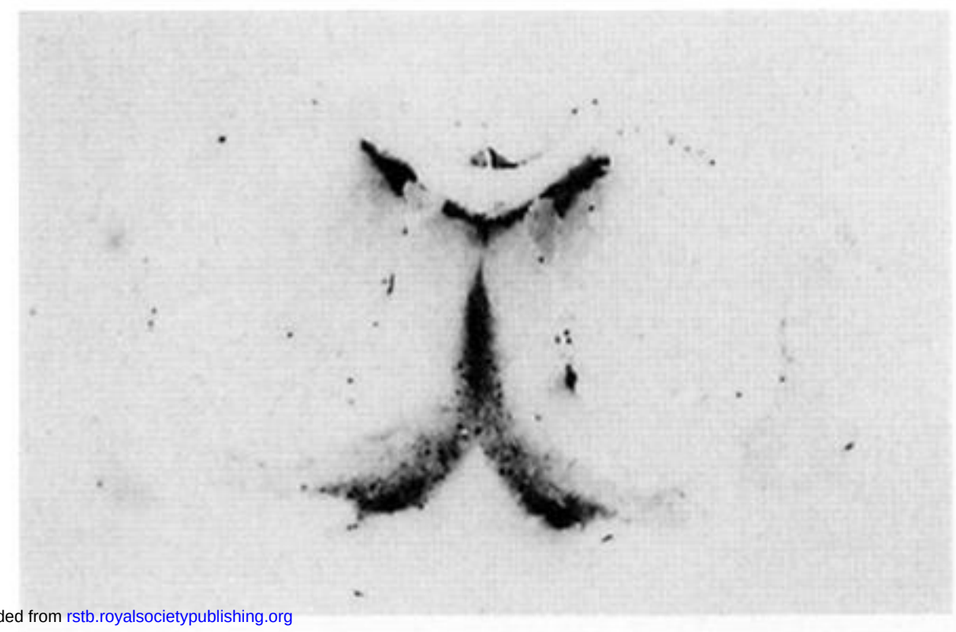
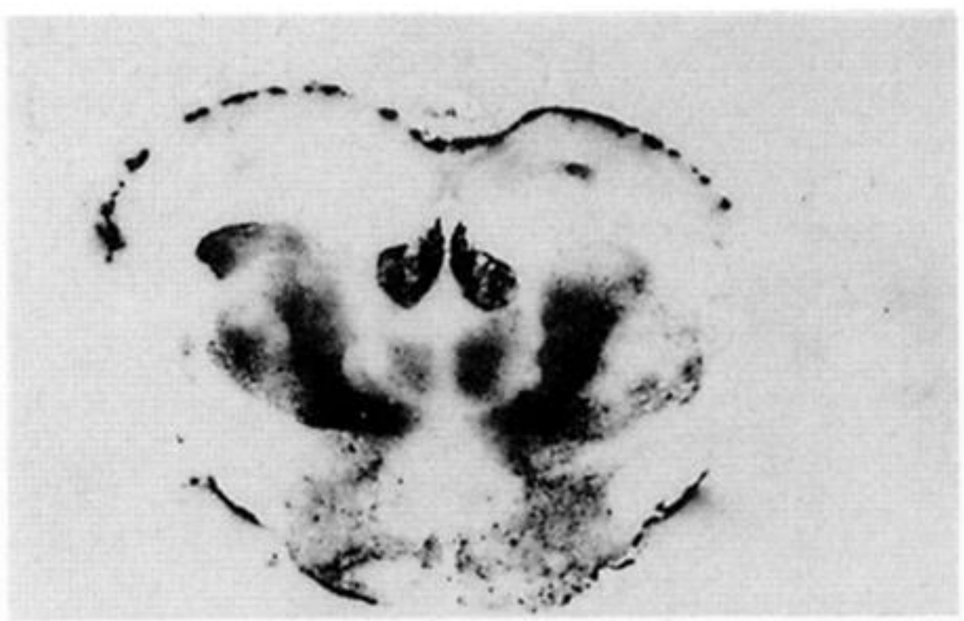
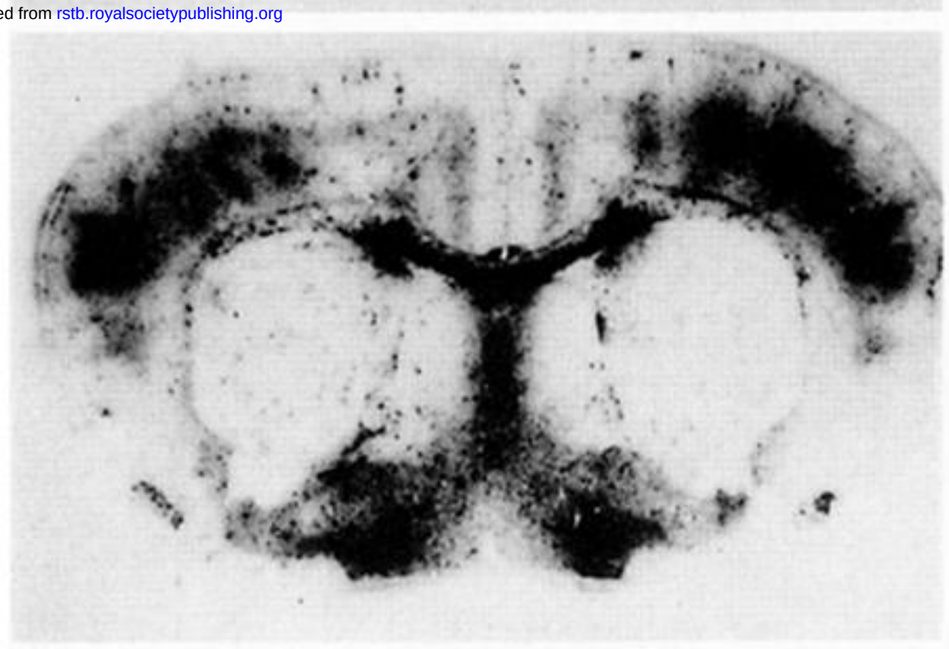
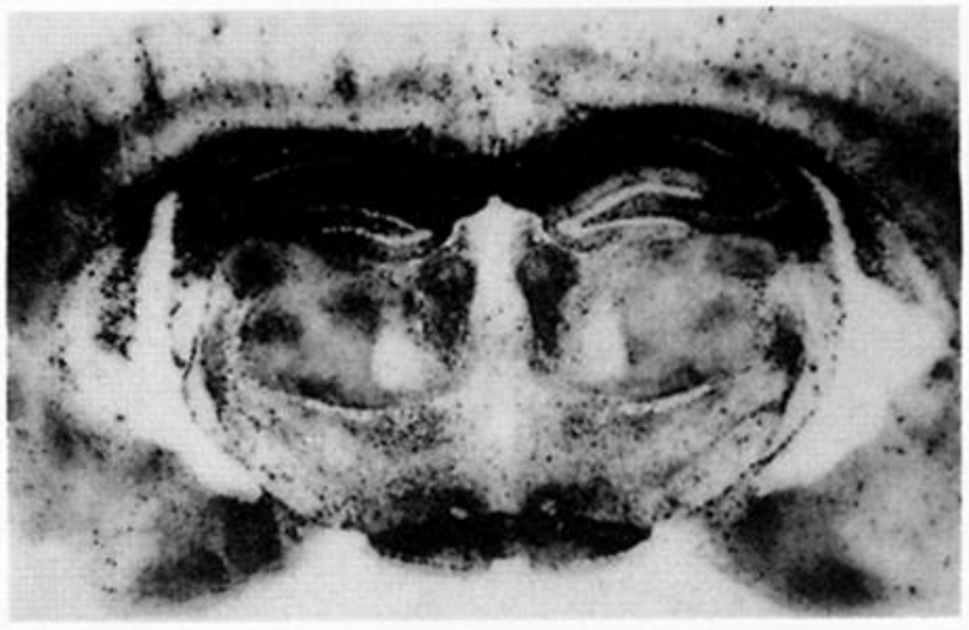


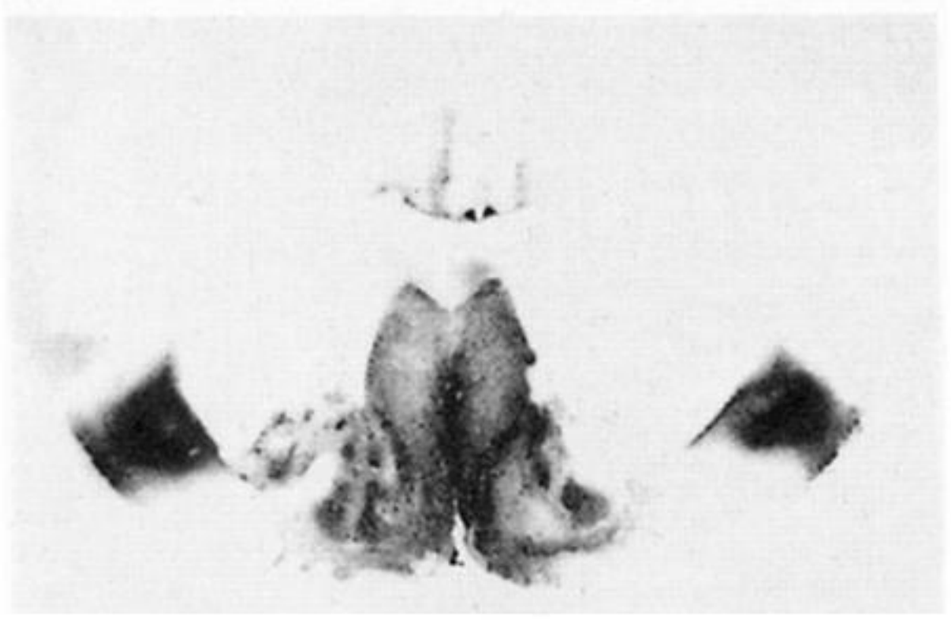


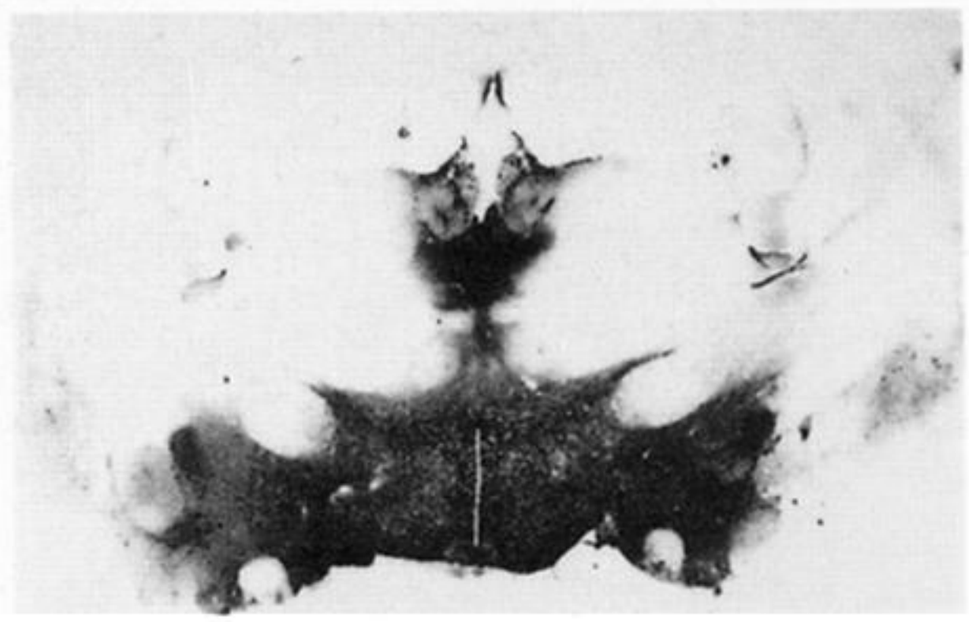
139H





Me7H





gure 3. The regional distribution of PrP^{Sc} in the brain revealed by histoblots is unique for each prion isolate. In is study, Tg(SHaPrP)-7 mice, which express high levels of $SHaPrP^{C}$, were inoculated in the thalamus with either :237, 139H or Me7H prions. Two levels of the brain are shown. Mice inoculated with Sc237 or 139H prions evelop clinical signs about 50 days after inoculation. For the Me7H isolate, animals develop clinical signs between 30 and 200 days. Am, amygdala; As, accumbens septi; Cd, caudate nucleus; dbB, diagonal band of Broca; Hp, ppocampus; Hy, hypothalamus; NC, neocortex; S, Septal nuclei; st, interstitial nucleus of the stria terminalis; ZI, na incerta. Thalamic nuclei in italics: *Hb*, habenula; *L*, lateral; *ML*, medial, pars lateralis; *MM*, medial, pars edialis; *Pv*, paraventricular; *VPL*, ventral posterior lateral; *VPM*, ventral posterior medial.

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gure 4. An artificial prion created in transgenic mice expressing a chimeric PrP^{C} produces a unique histoblot ttern of PrP^{Sc} accumulation in Syrian hamster. (*a*) Syrian hamsters were inoculated with a brain homogenate om Tg(MH2M) mice infected with RML prions. The most intense PrP^{Sc} signal occurs along the hippocampal sure in the hippocampus (also see figure 1) and subpially at the periphery of the cerebral hemispheres and alamus. The pattern is significantly different than that produced by Sc237 prions passaged among Syrian msters (*b*).

gure 5. Clinical signs in uninfected transgenic mice which express high levels of wild-type PrP^{C} are due to a crotizing myopathy (*a*) characterized by degeneration and phagocytosis of skeletal muscle. Vacuolar degeneration) and reactive astrocytic gliosis (*c*) in the CNS are confined to the stratum lacunosum moleculare (LM) of the ppocampus and are insufficient to account for clinical signs. P, pyramidal cell layer; R, stratum radiatum. Bar in) is 50 μm. Bar in (*c*) is 50 μm and also applies to (*b*). Haematoxylin and eosin stain (*a* & *b*), glial fibrillary acidic mmunohistochemistry (*c*).