Expression of cellular prion protein (PrPc) in the rat central nervous system

F.J. Moleres, M. Castle and J.L. Velayos

Departamento de Anatomía, Facultad de Medicina, Universidad de Navarra, Pamplona, Spain

SUMMARY

The localization of cellular prion protein in the central nervous system of adult rats is an important step not only for understanding its biology, but also to gain a better understanding of the propagation of pathogenic prion protein within the central nervous system in spongiform encephalopathies. Here, the expression of cellular prion protein is studied by immunocytochemistry and Western blotting techniques.

The presence of the protein varied in the rostrocaudal direction: it was less abundant in the brainstem than in prosencephalon. PrPc-positive cells were especially abundant in the cerebral cortex. The immunoreaction was present in areas projecting to the intermediate band of the mediodorsal thalamic nucleus and the anteroventral nucleus of the thalamus. Some immunopositive neurons, glial cells, and fibres were located around vessels in areas where nitrergic activity is well documented. Our findings lead us again to propose a retrograde propagation of altered prion proteins in human spongiform encephalopathies such as fatal familial insomnia, followed by an alteration of the nitrergic systems. Moreover, destruction of the mediodorsal thalamic nucleus in fatal familial insomnia may unleash an alteration of prion and nitrergic physiology.

Key words: Cellular prion protein – Central nervous system

INTRODUCTION

The localization of cellular prion protein (PrPc) in the brain is an important step for understanding prion biology and, moreover, the expression of PrPc is necessary for the subsequent replication of the pathological prion protein (PrPsc) in spongiform encephalopathies.

In fatal familial insomnia (FFI), a human spongiform encephalopathy that shows similarities to Creutzfeldt-Jakob's disease, anatomopathological alterations of the intermediate band of the thalamic mediodorsal nucleus (MD) and the anteroventral nucleus of the thalamic anterior complex (A) are consistently observed (Manetto et al., 1992; Gambetti et al., 1995; Dorandeu et al., 1998). The intermediate band of the MD and the anteroventral nucleus of the A complex are structures with a different connectivity from the other portions of the MD and the A and neighbouring thalamic nuclei (Velayos and Alfageme, 1999).

We have previously proposed for FFI a mainly retrograde propagation of the modified proteins from the MD (especially its intermediate band) and A (especially the anteroventral nucleus) to several areas of the central nervous system; for example, to nitrergic areas of the basal prosencephalon and brainstem (Velayos et al., 1998; Velayos and Alfageme, 1999). Some of these prosencephalic and brainstem cells are located in a perivascular situation (Velayos et al., 1998; Velayos and Alfageme, 1999). This, along with HRP - NADPH-diaphorase (NADPH-d) colocation in this type of neuron in cases of mediodorsal and anterior nuclei tracer injections, leads us to propose the possible pathophysiological involvement of nitrergic systems in FFI (Velayos et al., 1998; Velayos and Alfageme, 1999).

These ideas are based on previous studies in the cat central nervous system (Velayos et al., 2002) where cellular prion protein immunode-

Submitted: May 16, 2003
Accepted: July 25, 2003

Correspondence to:
J.L. Velayos. Departamento de Anatomía, Facultad de Medicina, Universidad de Navarra, C/ Irunlarrea s/n, 31008 Pamplona (Spain).
Phone: 948425600 ext. 6396. E-mail address: jvelayos@unav.es

tection varies in the rostrocaudal direction: the protein is less abundant in the brainstem than in the prosencephalon. Positive cells are especially abundant in the cerebral cortex. The immunoreaction is present in areas projecting to the intermediate band of the thalamic mediodorsal nucleus and to the anteroventral nucleus of the thalamus. Also, some of these cells are situated around vessels in the basal prosencephalon. Extrapolations are possible (Heckers et al., 1992) because the connectivities of the MD and A in the cat are similar to that of the primate.

Recently, the expression of PrPc in several areas of the central nervous system of the rat (Verghese-Nikolakaki et al. 1999) and mouse (Liu et al., 2001; Ford et al., 2002) have been studied.

In the present work we attempted to shed further light on earlier results, this being a continuation of work previously carried out by us. The study was performed in the central nervous system of rat, a less phylogenetically developed species than the cat. Thus, we hope that a comparative study of different species should allow us to understand the physiopathological processes of spongiform encephalopathies more accurately.

MATERIAL AND METHODS

Immunocytochemistry

Twelve sex –and age– matched Wistar rats were transcardially perfused with 100 ml of saline, followed by 350 ml of 4% paraformaldehyde. The whole brain was sectioned at 40 µm with a freezing microtome. Sections were treated to neutralize endogenous peroxidase. Free-floating sections were microwaved for 1 minute at maximum power, followed by 1 minute at minimum power, and cooled for 2 hours at room temperature. Sections were blocked with 5% normal goat serum and incubated for 48 hours at 4ºC with the primary antibody 6H4 (Prionics). Immunocytochemistry was performed employing the ABC method. DAB was used as chromogen. Some of the brains were counterstained with Nissl stain.

NOS immunocytochemistry was accomplished using the primary AB5380 antibody (Chemicon), followed by the PAP-method, adding DAB as chromogen.

Western Blotting

The brains of three Wistar rats were removed and carefully dissected. The different brain regions were immediately frozen in liquid nitrogen and stored at -80ºC until total protein measurements. 25 µg was loaded on a 15% polyacrylamide gel under reducing conditions. Proteins were transferred to a nitrocellulose membrane. The membrane was blocked with 10% defatted milk and the primary 6H4 antibody (Prionics) was incubated overnight at 4ºC. Immunoreactivity was developed with a peroxidase-conjugated secondary antibody and detection was performed with the Lumilight-plus kit (Roche).

The animals used for the experiments were deeply anaesthetized before both perfusion and decapitation.

RESULTS

PrPc immunodetection varied in the different brain regions in the rostrocaudal direction. The protein was less abundant in the brainstem than in the prosencephalon (Figure 1).

We observed a positive PrPc immnunoreaction in the cerebral cortex: this was more pronounced in the deep layers (layers V and VI) than in layer II (Figures 2 A, 2 B). We also observed immunoreaction in the hippocampus (CA1 field) (Figure 2 C), and in subcortical structures: namely, the olfactory bulb (Figure 3 C), septal region, preoptic area (Figure 2 E), accumbens nucleus (Figure 2 F), amygdala (Figures 3 A, 3 B), thalamus (Figure 2 D) and striatal complex. Some positive PrPc immunoreaction was observed in the Purkinje cells of the cerebellum (data not shown).

Positive neurons around vessels were observed in several zones (Figures 3 B, 3 C, 3 E).

Positive PrPc glial cells in a perivascular location were observed in several areas of the rat central nervous system. In addition, immunopos-

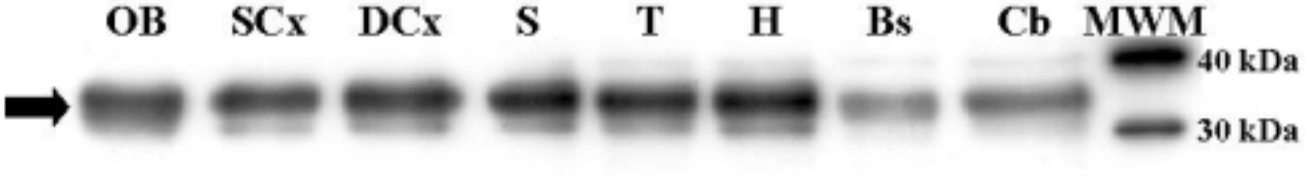

Figure 1.- Western Blot analysis of samples (25 µg protein) from the olfactory bulb (OB), superficial (SCx) and deep layers (DCx) of cerebral cortex, striatum (S), thalamus (T), hippocampus (H), brainstem (Bs) and cerebellum (Cb). Black arrow indicates PrPc. Note the lower expression of PrPc in brainstem and cerebellum as compared with rostrally located structures.

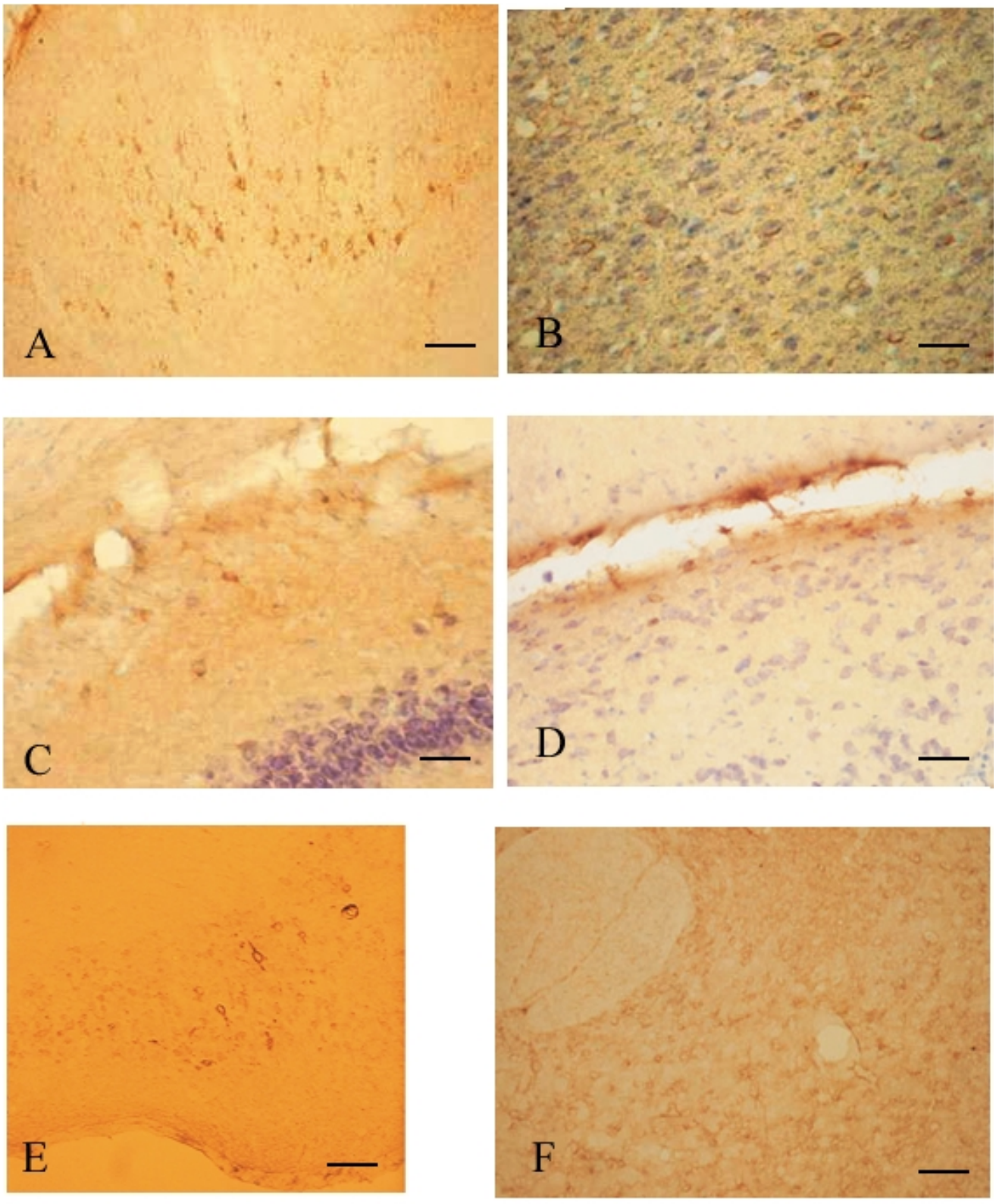

Figure 2.- PrPc-positive cells in deep layers of the frontal cortex (**A**, **B**), CA1 of hippocampus (**C**), laterodorsal thalamic nucleus (**D**), preoptica area (**E**) and accumbens nucleus (**F**). Calibration bar = 100 µm.

itive PrPc fibres were also observed in a perivascular situation (Figure 3 E).

By Western blotting, the band corresponding to PrPc was detected by the monoclonal antibody 6H4 at 35 kDa. Immunoblot analysis of PrPc in several samples of the central nervous system showed that there are fewer PrPc-positive cells in the brainstem than in rostral zones such as the cerebral cortex (Figure 1). By contrast, clearly positive NOS immunoreactive neurons were observed in the dorsal tegmental nucleus (Figure 3 D).

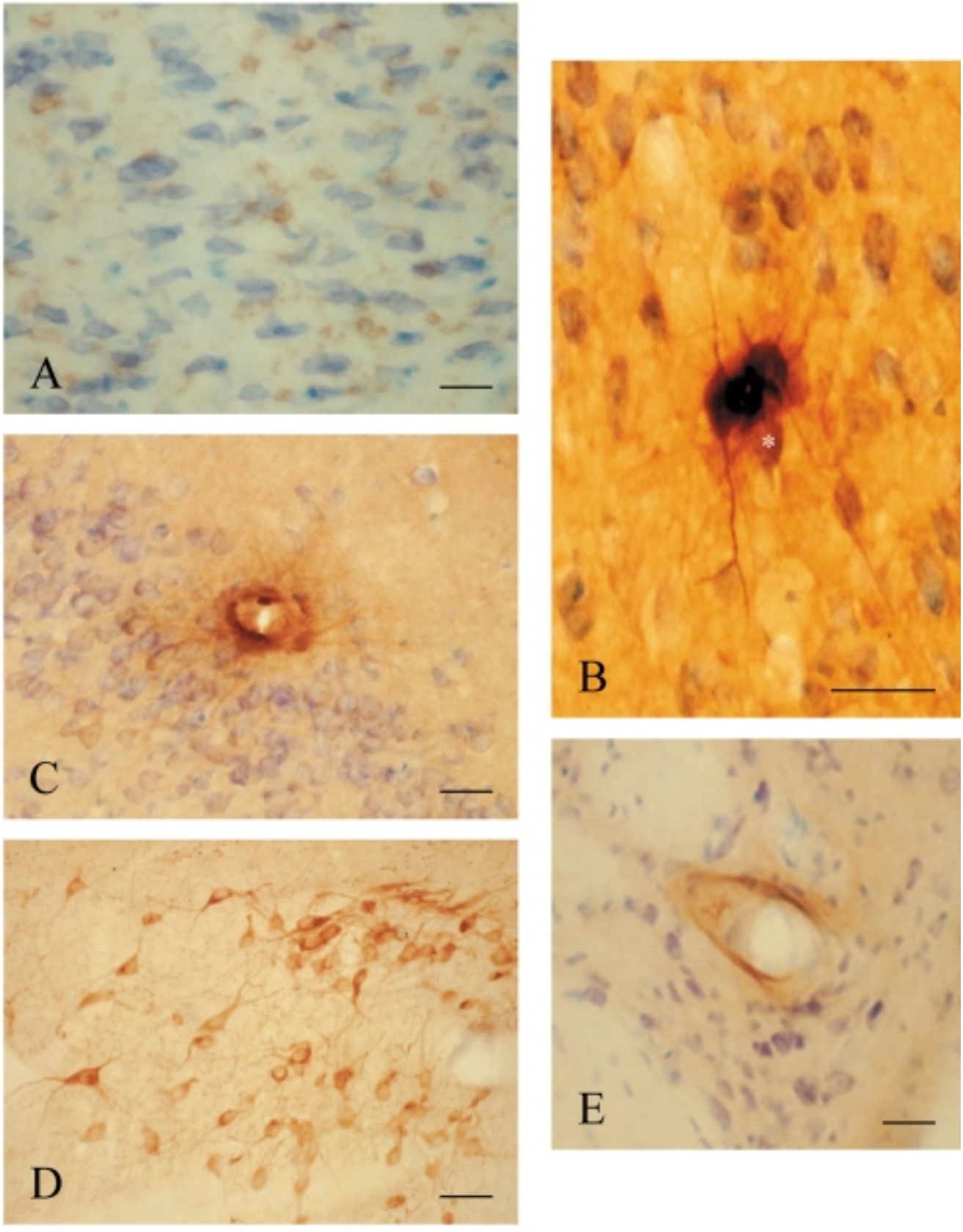

Figure 3.- PrPc immunopositivity in central amygdaloid nucleus (**A**, **B**), olfactory bulb (**C**) and peripeduncular nucleus (**E**). NOS-positive cells in dorsal tegmental nucleus (**D**). Note PrPc-positive glial cells (E), neurons (C) and fibres around vessels (C, E) and the close relationship between neurons and glia (star) (B). Calibration bar = 100 µm.

DISCUSSION

The anatomopathological findings of cases of FFI studied to date show that after some time the cerebral cortex is affected in its deep layers (Manetto et al., 1992; Parchi et al., 1995). We have previously observed retrograde labelling of these layers after tracer injections in the MD and A of the cat (Velayos and Reinoso-Suárez, 1985; Velayos et al., 1993; Velayos and Cruz, 1994; Velayos, 1997). Our previous works lead us to suspect that the propagation of the infective proteins from the MD and A to the cerebral cortex, and mainly to the cingulate and prefrontal cortices, must occur mainly through a retrograde pathway: that of corticothalamic connections (Velayos and Reinoso-Suárez, 1985; Velayos et al., 1993; Velayos and Cruz, 1994; Velayos, 1997; Velayos et al., 1998). Moreover, other authors (Divac and Passingham, 1980; Heckers et al., 1992; McGinty et al., 1994; Scott et al., 1992; Velayos et al., 1998; Beekes et al., 1996; Sàles et al., 2002) have suggested retrograde, rather than anterograde, propagation of prion proteins to subcortical structures.

Again in rats, we observed an abundant presence of immunoreactivity in deep layers of the cerebral cortex, and especially of the prefrontal cortex (Figures 2A, 2B). Thus, in this sense there is some similarity to the situation observed in cats. We also saw PrPc-positive neuronal bodies in subcortical structures projecting to the thalamus (Figures 2 E, 2 F, 3 A, 3 B, 3 E), confirming our previous findings in cats. Thus, according to the works cited there is a possible propagation of the infected prion proteins in a retrograde direction from the thalamus to these subcortical zones.

We have previously observed that some of the neurons around vessels projecting to the intermediate band of the MD and to the anteroventral nucleus of the A complex in the cat are nitrergic (Velayos and Alfageme, 1999; Velayos et al., 2002). Here, in the rat we observed PrPc-positive cells and fibres around vessels; some of them were of the neuronal type while others were glial cells (Figures 3 B, 3 C, 3 E). Accordingly, we are again tempted to speculate about a possible nitrergic involvement in the physiology and pathophysiology of prion proteins.

ACKNOWLEDGEMENTS

This work was supported by grants BMH4-CT96-856 (EU) and PIUNA 2002 (Plan de Investigación de la Universidad de Navarra, 2002).

Special thanks are due to Ms. María Ángeles Erdozain, Ms. Ainhoa Moreno, Mr. Pedro García and Mr. Rafael Heredero for their unvaluable technical support. Many thanks are also due to Mr. José Joaquín Hernández-Roca and Mr. Jordi Broncano for their help.

REFERENCES

BEEKES M, BALDAUF E and DIRINGER H (1996). Sequential appearance and accumulation of pathognomonic markers in the central nervous sytem of hamsters orally infected with scrapie. *J Gen Virol*, 77: 1925-1935.

DIVAC I and PASSINGHAM R (1980). Connections of the mediodorsal nucleus of the thalamus in the tree shrew. II. Efferent connections. *Neurosci Lett*, 19: 21-26.

DORANDEU A, WINGERTSMANN L, CHRÉTIEN F, DELISLE M-B, VITAL C, PARCHI P, MONTAGANA P, LUGARESI E, IRONSIDE JW, BUDKA H, GAMBETTI P and GRAY F (1998). Neuronal apoptosis in fatal familial insomnia. *Brain Path*, 8: 531-537.

FORD MJ, BURTON LJ, GRAHAM CH, FROBERT Y, GRASSI J, HALL SM and MORRIS RJ (2002). A marked disparity between the expression of prion protein and its message by neurones of the CNS. *Neuroscience*, 111: 533-551.

GAMBETTI P, PARCHI P, PETERSEN RB, CHEN SG and LUGARESI E (1995). Fatal familial insomnia and familial Creutzfeldt-Jakob disease: clinical, pathological and molecular features. *Brain Pathology*, 5: 43-51.

HECKERS S, GEULA C and MESULAM M (1992). Cholinergic innervation of the human thalamus: dual origin and differential nuclear distribution. *J Comp Neurol*, 325: 68-82.

LIU T, ZWINGMAN T, LI R, PAN T, WONG B-S, PETERSEN RB, GAMBETTI P, HERRUP K and SY M-S (2001). Differential expression of cellular prion protein in mouse brain as detected with multiple anti-PrP monoclonal antibodies. *Brain Res*, 896: 118-129.

MANETTO V, MEDORI R, CORTELLI P, MONTAGNA P, TINUPER P, BARUZZI A, RANCUREL G, HAUW J, VANDERHAEGHEN J, MAILLEUX P, BUGIANI O, TAGLIAVINI F, BOURAS C, RIZZUTO N, LUGARESI E and GAMBETTI P (1992). Fatal familial insomnia: clinical and pathologic study of five new cases. *Neurology*, 42: 312-319.

MCGINTY D, SZYMUSIAK RS, KRILOWICZ B and MORAIRTY S (1994). Thermoregulatory control of NREM sleep. In: Guilleminault C, Lugaresi E, Montagna P, Gambetti P (eds). *Fatal Familial Insomnia: Inherited Prion Diseases, Sleep, and the Thalamus*. Raven Press, New York, pp 101-108.

PARCHI P, CASTELLANI R, CORTELLI P, MONTAGNA P, CHEN S, PETERSEN R, MANETTO V, VNENCAK-JONES C, MCLEAN M, SHELLER J, LUGARESI E, AUTILIO-GAMBETTI L and GAMBETTI P (1995). Regional distribution of protease-resistant prion protein in fatal familial insomnia. *Ann Neurol*, 38: 21-29.

SÀLES N, HASSIG R, RODOLFO K, DI GIAMBERARDINOP L, TRAIFFORT E, RUAT M, FRETIER P and MOYA KL (2002). Developmental expression of the cellular prion protein in elongated axons. *Eur J Neurosci*, 15: 1163-1177.

SCOTT JR, DAVIES D and FRASER H (1992). Scrapie in the central nervous system: neuroanatomical spread of infection and Sinc control of pathogenesis. *J Gen Virol*, 73: 1637-1644.

VELAYOS JL (1997). Laminar and areal distribution of corticothalamic neurons with contralateral projection. *Eur J Anat*, 1: 105-106.

VELAYOS JL and ALFAGEME F (1999). Forebrain and brainstem perivascular neurons projecting to the thalamus (An anatomical explanation of the pathophysiology of fatal familial insomnia). *Eur J Anat*, 3: 87-92.

VELAYOS JL, CASAS-PUIG R and REINOSO-SUAREZ F (1993). Laminar organization of the cortical projections to the intralaminar and medial thalamic nuclei in the cat. In: Minciacchi D, Molinari M, Macchi G, Jones EG (eds). *Thalamic Networks for Relay and Modulation*. Pergamon Press, New York, pp 185-195.

VELAYOS JL and CRUZ J (1994). Afferent projections to the mediodorsal and anterior thalamic nuclei. In: Guilleminault C, Lugaresi E, Montagna P, Gambetti P (eds). *Fatal Familial Insomnia: Inherited Prion Diseases, Sleep, and the Thalamus*. Raven Press, New York, pp 57-69.

VELAYOS JL, OLIVA M and ALFAGEME F (1998). Afferent projections to the mediodordal and anterior thalamic nuclei in the cat. Anatomical - clinical correlations. *Brain Path*, 8: 549-552.

VELAYOS JL and REINOSO-SUÁREZ F (1985). Prosencephalic afferents to the mediodorsal thalamic nucleus. *J Comp Neurol*, 242: 161-181.

VELAYOS JL, ULLÁN J, AMAT A, RAMOS P, SIEIRA JA and SIEIRA R (2002). Expression of cellular prion protein (PrPc) in the cat central nervous system. Some findings. *Eur J Anat*, 6: 23-29.

VERGHESE-NIKOLAKAKI S, MICHALÑOUDI H, POLYMENIDOU, GROSCHUP MH, PAPADOPOULOS GC and SKALAVIADIS T (1999). Expression of the prion protein in the rat forebrain - an immunohistochemical study. *Neurosci Lett*, 272: 9-12.