# Expression of cellular prion protein (PrPc) in the cat central nervous system. Some findings

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## SUMMARY

The expression of cellular prion protein in the central nervous system of normal cats and in cases of kainic acid injections into the mediodorsal thalamic nucleus were studied. Cellular prion protein immunodetection varied in a rostrocaudal direction: the protein was less abundant in the brainstem than in the prosencephalon. Especially abundant were the positive cells in the cerebral cortex. The immunodetection was present in areas projecting to the intermediate band of the thalamic mediodorsal nucleus and to the anteroventral nucleus of the thalamus. Some of these cells were situated around vessels in the basal prosencephalon.

The immunodetection was less abundant in cases of kainic acid lesions.

NAPPH-diaphorase activity was also studied. In control cases, we observed positive NADPH-diaphorase cells around vessels, especially in the basal prosencephalon. The amounts of such cells and the intensity of the reaction was lower after kainic acid lesions.

Our findings lead us to propose a retrograde propagation of altered prion proteins in human spongiform encephalopathies such as fatal familial insomnia; a possible alteration of nitrergic systems is possible. Moreover, there is possibly an alteration of the prion and nitrergic physiology simply due to the destruction of the thalamic mediodorsal nucleus in fatal familial insomnia.

**Key Words:** Cellular prion protein – Nitrergic systems – Spongiform encephalopathies

## INTRODUCTION

In fatal familial insomnia (FFI), a 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 MD and the anteroventral nucleus of the A complex (MDi, Av) are structures with a different connectivity from the other portions of MD and A and the neighboring thalamic nuclei (Velayos and Alfageme, 1999).

After some time, other structures such as the deep layers of the cerebral cortex are also affected in FFI (Manetto et al., 1992; Parchi et al., 1995). The most affected area of the cerebral cortex is the cingulate cortex, and the least, the occipital cortex (Parchi et al., 1995; Cortelli et al., 1997). The disconnection of the limbic and paralimbic cortex and the hypothalamus due to the degeneration of the limbic thalamus could be responsible for the imbalance observed in FFI (Lugaresi and Montagna, 1994).

We have previously proposed for FFI a mainly retrograde propagation of the modified proteins from 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

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in a perivascular situation (Velayos et al., 1998; Velayos and Alfageme, 1999). This fact, along with HRP – NADPH-diaphorase (NADPH-d) collocation in this type of neurons in cases of mediodorsal and anterior nuclei tracer injections leads us to propose a possible pathophysiological implication of nitrergic systems in FFI (Velayos et al., 1998; Velayos and Alfageme, 1999).

Salès et al. (1998) have described cellular prion protein (PrPc) localization in rodent and primate brain. No description of PrPc localization in cats has been made. Recently, Esiri et al. (2000) have studied PrPc immunoreactivity in human brain samples.

Extrapolations are possible (Heckers et al., 1992) because the connectivity of MD and A in the cat is similar to that of the primate. On the other hand, the localization of PrPc in the brain is an important step to understand prion biology, and moreover, the expression of PrPc is necessary for the subsequent replication of the pathological prion protein (PrPsc). Accordingly, in the present study we were prompted to attempt to pinpoint the localization of PrPc in the central nervous system of the cat (mainly in the basal prosencephalon and brainstem). Moreover, we performed kainic acid lesions in the intermediate band of MD in order to study the consequences in nitrergic areas, and also the consequences for PrPc localization (mainly in the basal prosencephalon and brainstem).

## Material and Methods

Adult cats of both sexes were used.

PrPc immunodetection was carried out in frozen sections of 40 and 50 µm of four cats brains. Sections were treated to neutralize endogenous peroxidase. MAB1562 (Chemicon) was the primary antibody used. The results shown here were obtained at dilutions of 1:5000. Diaminobenzidine was used as a chromogen.

NADPH-d histochemistry was performed in frozen sections of 40-50 µm from the same four cat brains. We used NADPH-d (Sigma) at a dilution of 83 mg./100 cc of TRIS solution (pH 7.2), adding Nitroblue Tetrazolium (NBT, Sigma) at a dilution of 82 mg./100 cc. The reaction temperature was of 36-37ºC.

Stereotaxic injections of 200-250 nl of kainic acid (5 nmol of kainic acid in 1 µl of 0.9% NaCl, pH 7.2) into the mediodorsal thalamic nucleus of three cats were performed (1 hour injection duration). 10 days after the surgical interventions, we studied the NADPH-d histochemistry mainly in basal prosencephalon and brainstem. In 2 cases, the immunodetection of PrPc was performed, paying special attention to the basal prosencephalon and brainstem.

Animals were always anaesthetized before surgery and perfusion.

## Results

*PrPc immunodetection*

PrPc immunodetection varied in the different brain regions in a rostrocaudal direction. The protein was less abundant in the brainstem than in the prosencephalon. Cell bodies were revealed as blank profiles in the deep layers of the cerebral cortex (Figure 1), the reticular thalamic nucleus (Figure 2), the basal prosencephalon (mainly in the substantia innominata, preoptic area and medial septum) (Figure 3), the parabrachial regions, the superior colliculus, the oral pontine reticular nucleus (Figure 4). Some cells were situated around vessels in the basal prosencephalon. Positive perivascular cells were observed in the substantia innominata, the lateral preoptic area and the medial septum (Figure 3).

In the cases of kainic acid injections in the MD, a lower amount of PrPc was observed in the cingulate and frontal cortices and in the basal prosencephalon; PrPc detection in the brainstem was similar to the control cases.

*NADPH-d histochemistry*

We observed positive NADPH-d cells around vessels, especially in the basal prosencephalon and brainstem (Figure 5), in agreement with previous findings (Velayos et al., 1998; Velayos and Alfageme, 1999).

After kainic acid injections into the MD, and in comparison with cases without lesions, we observed a less pronounced reaction and a lower number of NADPH-d positive cells (Figure 6) in the basal prosencephalon (mainly in the medial septum, diagonal band of Broca and preoptic area); this decrease was less marked in the brainstem.

## 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 (Velayos and Reinoso-Suárez, 1985; Velayos et al., 1993; Velayos and Cruz, 1994; Velayos, 1997).

Anatomopathological findings have also shown an important affectation of the cingulate cortex (Parchi et al., 1995), and this cortex projects to the MD and A (Velayos et al., 1993; Velayos and Cruz, 1994). The least affected cortex is the occipital one (Parchi et al., 1995;

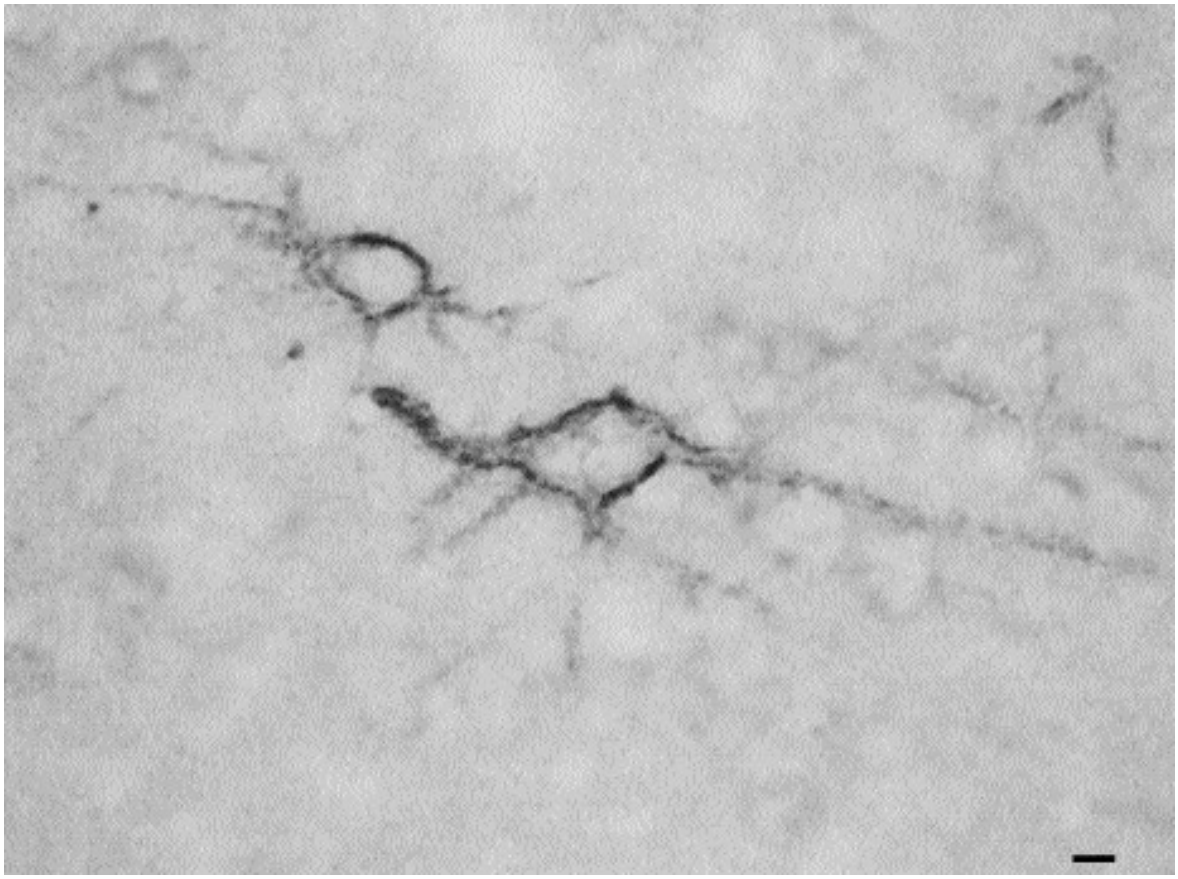

**Figure 1** .- PrPc-positive cells in frontal cortex. Note superficial immunoreaction. Scale bar: 50 µm.

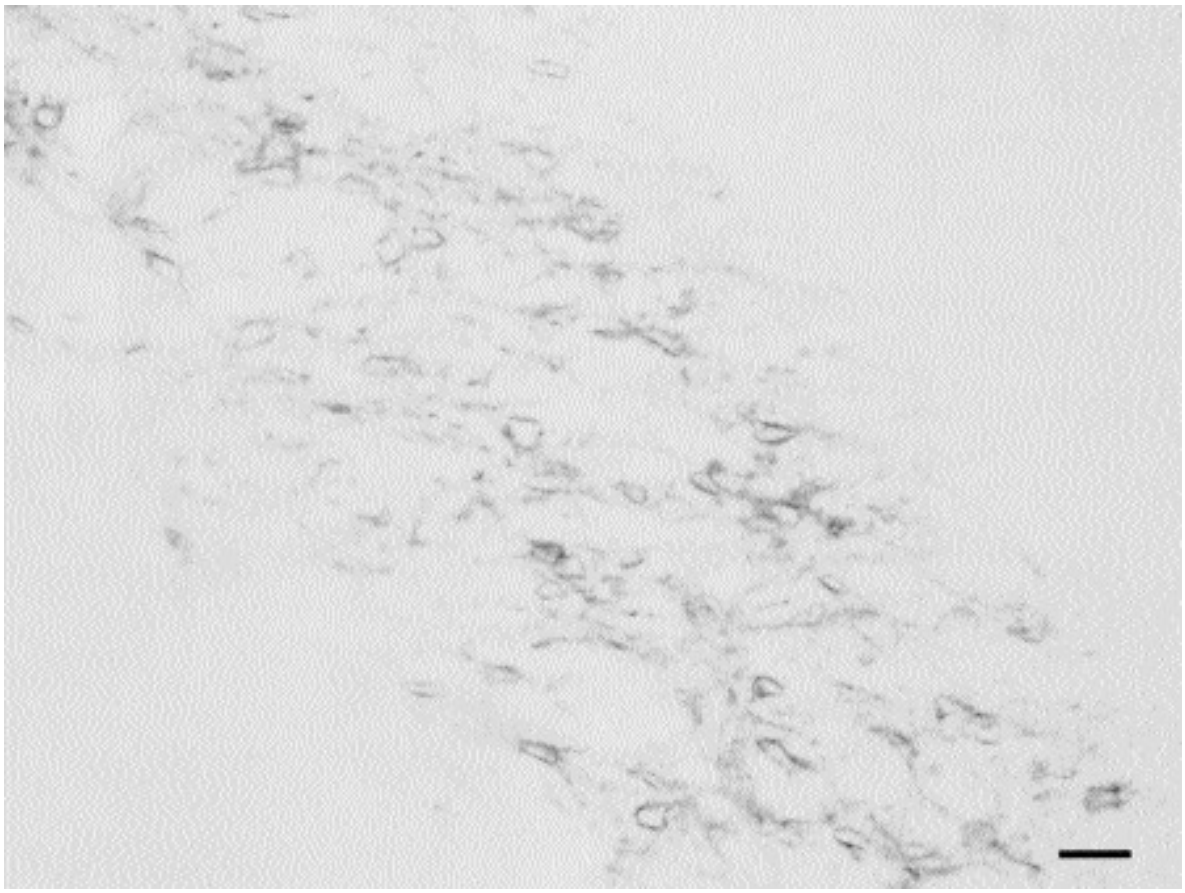

**Figure 2** .- PrPc-positive cells in the reticular thalamic nucleus. Scale bar: 50 µm.

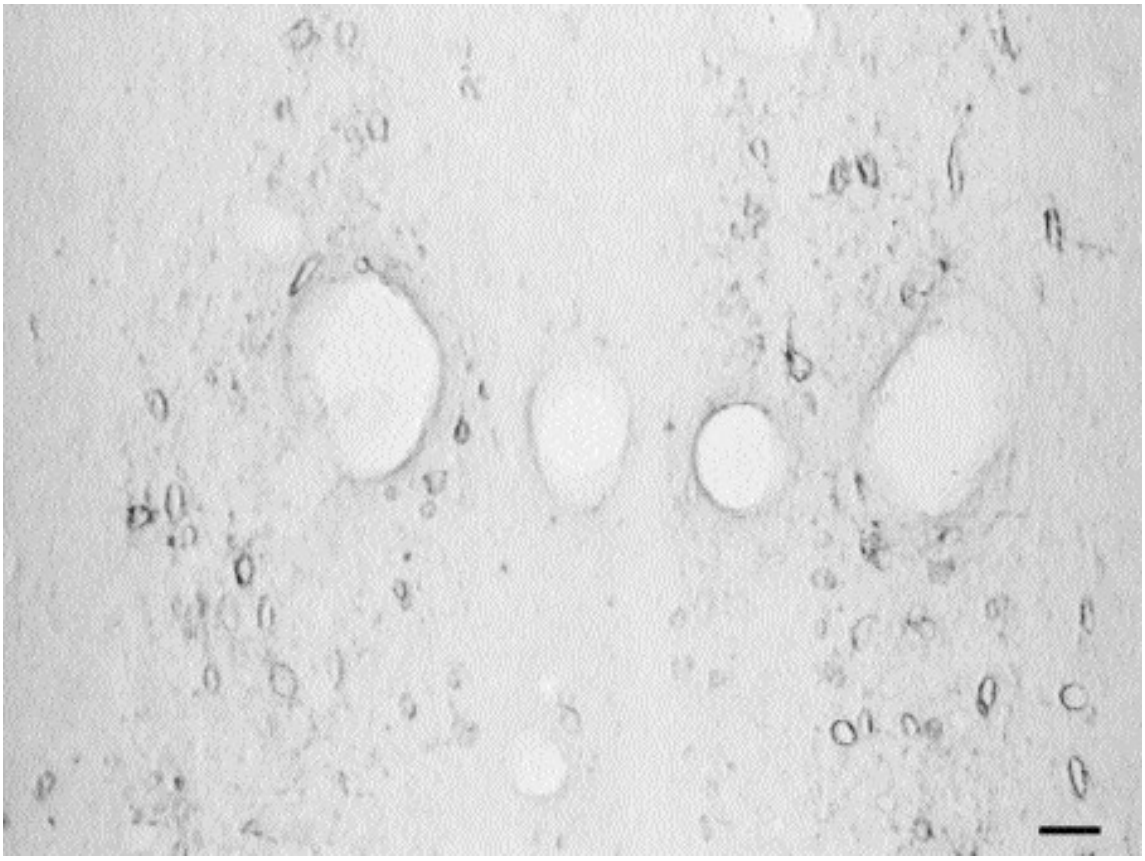

**Figure 3** .- PrPc-positive cells in the medial septum (some of them around vessels). Scale bar: 50 µm.

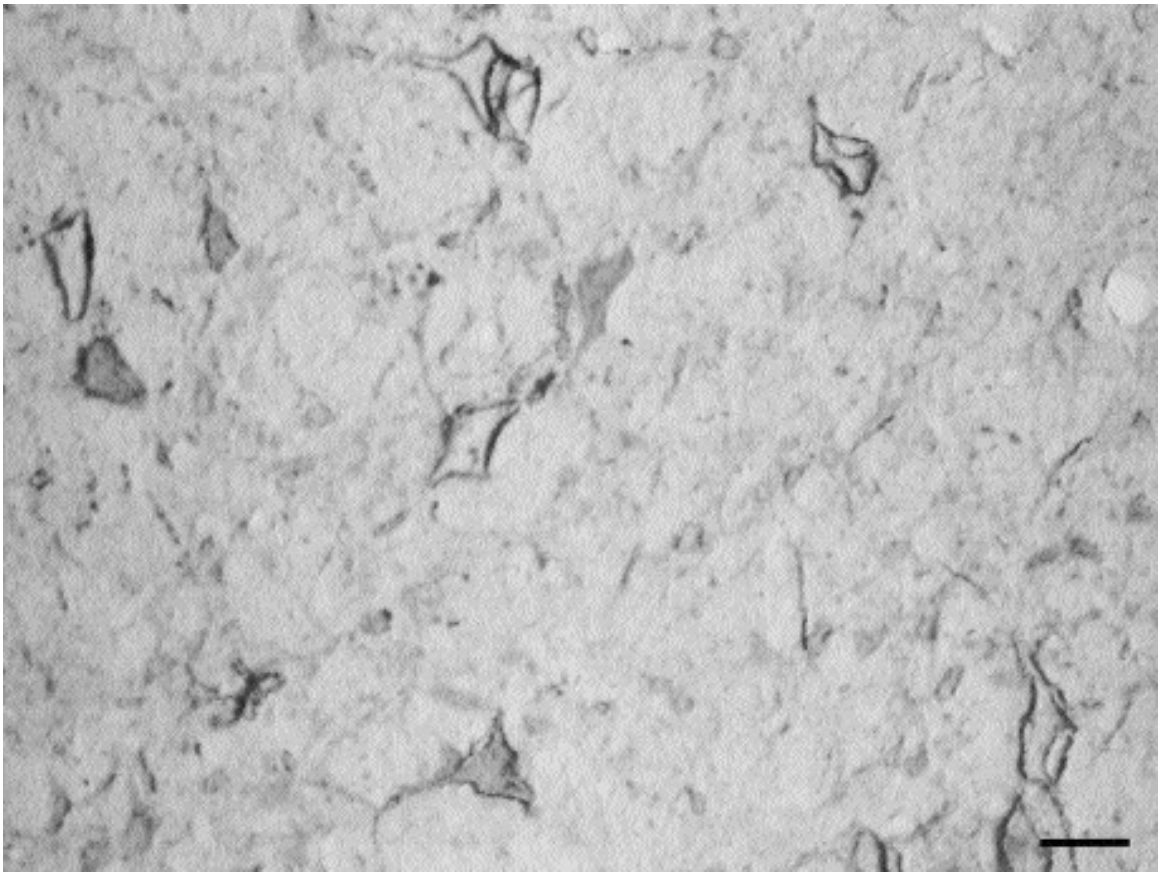

**Figure 4** .- PrPc-positive cells in the oral pontine reticular nucleus. Scale bar: 50 µm.

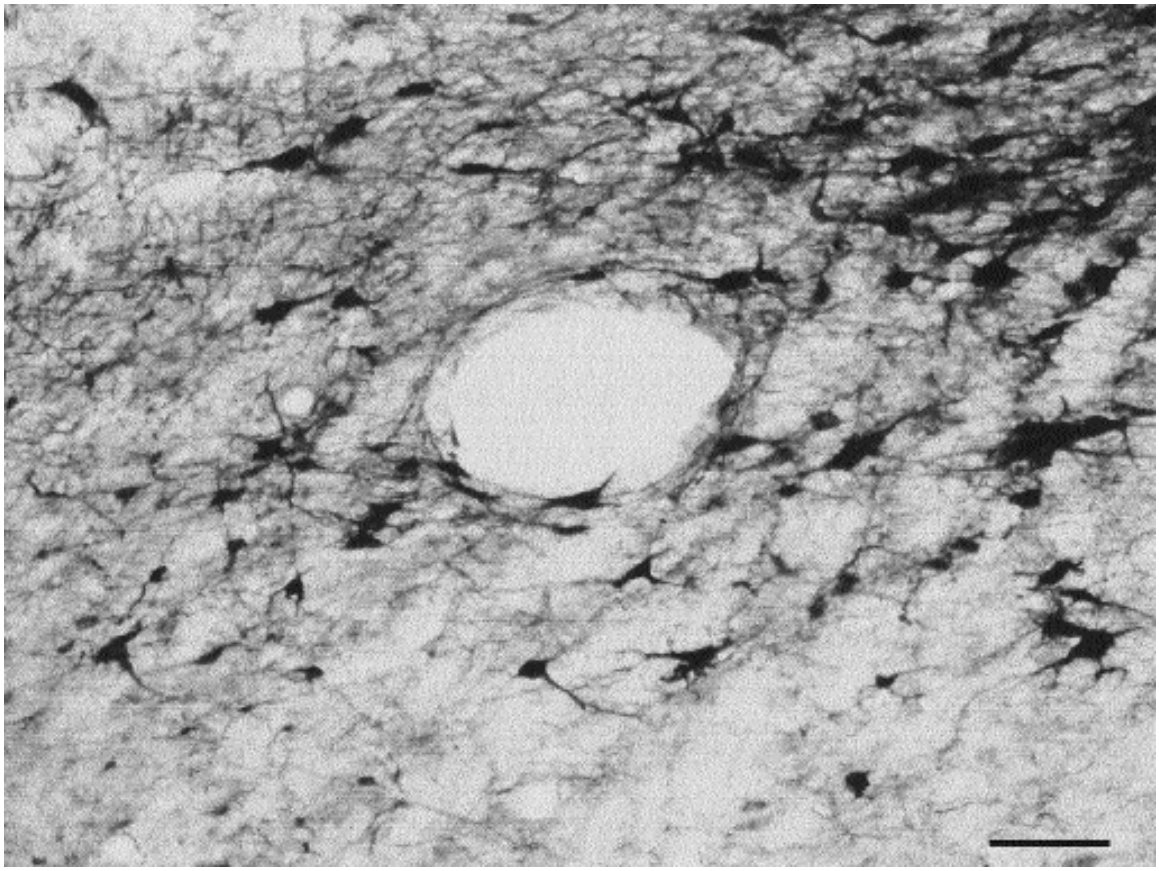

**Figure 5** .- Brainstem NADPH-diaphorase-positive cells around a vessel. Scale bar: 50 µm.

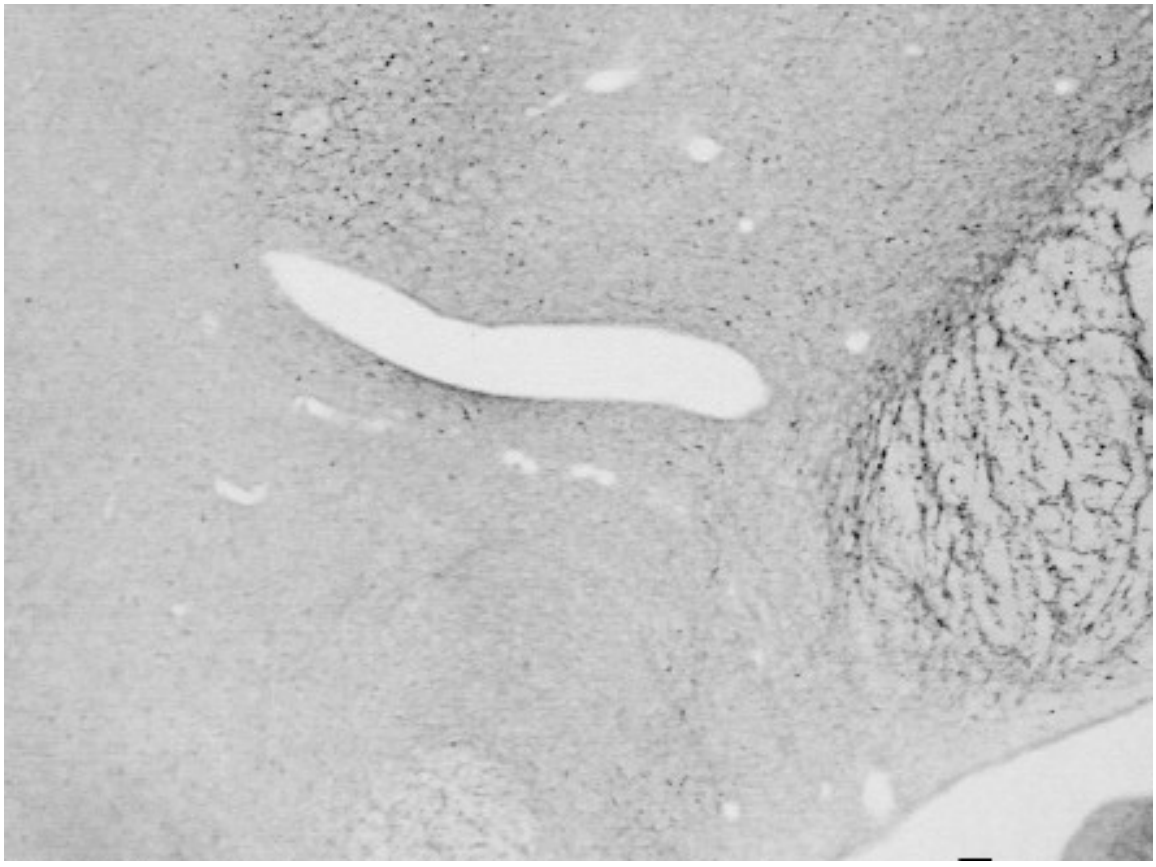

**Figure 6** .- Note decrease in the NADPH-diaphorase reaction after a kainic acid lesion in the thalamus (see text). Scale bar: 50 µm.

Cortelli et al., 1997), and this area scarcely projects to the MD and A (Velayos et al., 1993; Velayos and Cruz, 1994). Several anatomical findings 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 findings (Divac and Passingham, 1980; Heckers et al., 1992; McGinty et al., 1994) have also suggested a more prominent propagation in a retrograde than in an anterograde way of the prion proteins to subcortical structures (Velayos et al., 1998).

PrPc immunodetection of the control cases was observed around cell bodies situated in areas projecting to the MDi and Av (Figures 1, 2, 3 and 4): deep layers of the cerebral cortex (including the occipital cortex), medial septum, preoptic area, substantia innominata, reticular thalamic nucleus, and, at lower intensity, brainstem regions such as the parabrachial areas and the oral pontine reticular nucleus. It is difficult to unravel the meaning of these findings. It is thought that normal prion proteins are involved in synaptic function. Nevertheless, their physiological meaning remains obscure (Brown, 2001). On the other hand, alterations of the circadian activity rhythms and sleep in mice devoid of prion protein have been reported (Tobler et al., 1996). It is possible that the propagation of altered proteins from MD and A to the neuronal bodies projecting to these thalamic nuclei might originate alterations of PrPc in the structures connecting to the thalamus, and consequently, alterations in their physiology.

The decrease in the intensity of the NADPH-d reaction and in the number of NADPH-d positive cells (Figure 6) in the basal prosencephalon after kainic acid injections into the MD suggests that the lesion elicited by the altered proteins in FFI within the thalamus could lead to an alteration of the nitrergic structures of the basal forebrain. In Alzheimer's disease, an increase in NADPH-d neurons in the substantia innominata has been observed (Benzing and Mufson, 1995). Moreover, in the plaques seen in patients suffering from Alzeimer's disease PrPc is expressed in the extracellular spaces (Ferrer et al., 2001). However, in FFI there is probably a decrease in the number of NADPH-d neurons of the basal forebrain due to the death of target thalamic cells.

Some of the neurons around vessels projecting to MDi and Av are nitrergic (Velayos and Alfageme, 1999). In the present experiments, the amount of positive PrPc cells and also the amount of positive NADPH-d cells close to vessels were lower after kainic acid MD injections than in control cases. These changes were observed mainly in the basal prosencephalon. Thus, there may also be an alteration in "prionic" and nitrergic physiology simply due to the destruction of the MD. In fact, the basal prosencephalon is related to the regulation of multiple biological cycles (Alam y Mallick, 1994).

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