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Cortical neuronal cytoskeletal changes associated with FIV infection

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HIV-1 infection is often complicated by central nervous system (CNS) dysfunction. Degenerative neuronal changes as well as neuronal loss have been documented in individuals with AIDS. Feline immunodeficiency virus (FIV) infection of cats provides a model for both the immune and the central nervous system manifestations of HIV infection of humans. In this study we have examined neurons in the frontal cortex of feline immunodeficiency virus-infected cats and controls for immunoreactivity with SMI 32, an antibody recognizing a non-phosphorylated epitope on neurofilaments. We noted a significant increase in the number of immunoreactive pyramidal cells in infected animals compared to controls. The changes seen in the neuronal cytoskeleton as a consequence of the inoculation with FIV were similar to those seen in humans undergoing the normal aging process as well as those suffering from neurological diseases, including Alzheimer's and dementia pugilistica. The changes we noted in the feline brain were also similar to that reported in animals with traumatic injuries or with spontaneously occurring or induced motor neuron diseases, suggesting that the increase in reactivity represents a deleterious effect of FIV on the central nervous system.

Keywords: FIV; brain; neuron; neurofilament; AIDS

Introduction

Infection with human immunodeficiency virus type 1 (HIV-1) is often complicated by the development of cognitive and motor abnormalities. A severe form of central nervous system dysfunction, the AIDS dementia complex, is associated with abnormalities in behavioral and cognitive functions and histopathological changes in the gray and white matter of the brain (Masliah *et al*, 1992a,b; Price *et al*, 1988; Price, 1994). These neuropathological changes are especially profound in the frontal and temporal lobes (Masliah *et al*, 1992a,b). In order to understand this disease and to develop strategies to ameliorate its pathologies, several animal models have been developed. We have chosen the cat/feline immunodeficiency virus (FIV) (Hurtrel *et al*, 1992; Lafrado *et al*, 1993; Henriksen *et al*, 1995) model to study the effects of HIV on the human brain for several reasons: (1) both FIV and HIV-1 are lentiviruses and share many common structural and biochemical properties; (2) the clinical syndrome for FIV and HIV-1 is remarkably similar; (3) from our previous work as well as the contributions of other investigations, the FIV/cat system has been developed into a reproducible disease model, with the establishment of reliable molecular markers, detectable as early as 3 months post-infection, of both general and neurological disease progression; (4) specific pathogen-free cats are available, eliminating confounding interactions with other feline pathogens; and (5) since cats are a species of veterinary importance and are commonly used as experimental animals in neuroscience, the physiology and anatomy of the feline brain are well characterized and accessible for comparative examination. The focus of this study was on the cerebral cortex of the cat which readily lends itself to quantitative analysis.

Recent studies have shown that the neurofilament proteins are sensitive markers for neurodegenerative diseases in humans (Schlaepfer, 1987; Hof *et al*, 1992; Masliah *et al*, 1993; Price, 1994;

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Trojanowski *et al*, 1993) and in animals (Cork *et al*, 1988; Higgins *et al*, 1983; Rousseaux *et al*, 1985; Shields, 1983). In this study we have examined the consequences of inoculation with FIV on the neurofilaments in the neuronal cytoskeleton. Our goals were to: (1) determine if the neurofilaments were affected; (2) to identify the neurons affected; (3) to determine which anatomic areas and layers contain labeled cells; and (4) to assess whether the changes seen correlate to CNS deficits found in FIV or HIV infection.

We and others have shown that cats infected with FIV develop numerous clinical sequelae, including peripheral lymphadenopathy, anisocoria, delayed righting and pupillary reflexes, delayed auditory and visual evoked potentials, slowed spinal and peripheral nerve conduction velocities, and altered sleep architecture (Lafrado *et al*, 1993; Phillips *et al*, 1994, 1996; Henriksen *et al*, 1995). In this study we document the effects in the frontal lobe of infection with the Maryland strain of FIV and compare and contrast our findings with those in HIV-infected humans. Using immunocytochemical methods which examine the neuronal cytoskeleton proteins, we observe abnormal neuronal profiles in the frontal lobe of the infected cats. We propose this feline/FIV system as a useful model to test therapeutically strategies to combat the effects of the HIV-1 on the CNS.

Results

Clinical evaluation

As previously reported, these FIV-infected animals followed an established pathway of both physical and neurological abnormalities (Phillips *et al*, 1994; Henriksen *et al*, 1995). The physical abnormalities were primarily lymphadenopathy and rough hair coat. The neurological abnormalities included delayed pupillary reflex, delayed righting reflex and anisocoria. There were also delays in the auditory and visual evoked responses. Sleep studies revealed that the FIV-infected cats spent more time awake than the controls and in addition showed a substantial reduction of rapid eye movements (REM).

Histopathological analysis

Although a perivascular mononuclear cell infiltrate and glial nodules are occasionally seen in the brains of FIV-infected animals (Hurtrel *et al*, 1992; Phillips *et al*, 1994; Boche *et al*, 1996), the frontal cortex of the animals analyzed here were free from these lesions. Additional immunocytochemical studies did not reveal the presence of infiltrating or activated macrophages in the frontal cortex. Our analysis then focused on the cortical neurons. In order to assess whether differences in neurofilament reactivity may reveal neuronal changes induced by FIV infection, immunocytochemical staining of neurofilaments was then performed.

Number of SMI 32 immunocytochemically labeled cells/cortical area

Normal controls Two animals (#10 and 14) served as controls. Four distinct regions of the frontal cortex were studied by immunocytochemical staining for SMI 32, which recognizes a non-phosphorylated epitope in neurofilament proteins (Sternberger and Sternberger, 1983). This monoclonal antibody was chosen since reactivity is sensitive to alteration in neurofilaments in humans and non-human primates (Hof *et al*, 1992; Siegal *et al*, 1993). The results of the analysis in these two control animals are seen in Table 1. The staining was filamentous and was located either perinuclear or in patches in the apical and basilar dendrites (Figure 1A).

The number of neurons labeled by the SMI 32 antibody was 1.2–2.9%. The labeled neurons were found nearly equally distributed between the supragranular layer iii and infragranular layer v. The mean number and percentage of labeled cells in animal 10 did not differ significantly from those in animal 14 (Table 1). The labeled cells varied in area from the smallest (350 μ^2) to the largest (2488 μ^2) pyramidal cells.

FIV infected cats In all the animals in the infected group (#9, 11, 17, 19) we noted a significant increase in the staining of the neurofilaments in pyramidal cells from the motor, premotor, prefrontal and anterior cingulate cortexes relative to the uninfected controls (Table 1). The staining of some of the cells in the FIV treated groups was so dense that in some instances the filaments appeared to nearly fill the cell body (Figure 1C, D, and Figure 2).

The labeled neurons were most numerous in layers iii and v, with very few stained cells in layers ii and vi (Table 1). The percent of cells stained in each cortical region of the FIV-inoculated animals, ranging from 7–20%, was significantly higher than that found in the controls (<3%, Table 1). In three of the four experimental animals (#9, 11 and 19), the largest percentage of stained cells was found in motor area 4, whereas in the fourth experimental animal (#17), premoter area six contained the most stained cells. On average, these two motor areas exhibited almost a ninefold increase in number of cells staining in the infected animals, whereas prefrontal area 12 and anterior cingulate area 32 showed an approximate fourfold increase in staining. Regardless of the area studied, all of the FIV-infected cats exhibited an increase in number and percentage of neurons staining in all regions examined, with slight differences between the animals. In both control and experimental animals, the greatest number of labeled cells was in layers iii and v and the cell size ranged from 407 μ^2 to 2099 μ^2.

Discussion

Due to developments in molecular and cellular biology over the past decade it has finally become possible to investigate the neuronal cytoskeleton. The role of the neurofilament proteins in maintenance of neuronal contours is well understood (Bersjadsky and Vasiliev, 1988; Mitchison and Kirschner, 1988). However, the role of the neurofilament protein in disease is just now being elucidated

Table 1 Percent of pyramidal cells stained with the SMI antibody

Animal Group	*Motor Area 4*	*Premotor Area 6*	*Prefrontal Area 12*	*Ant. Cingulate Area 32*
Normal Control #10	6/379 1.6% iii–4 v–2	9/485 1.9% iii–5 v–4	10/425 2.4% iii–5 v–4 vi–1	9/395 2.3% iii–4 v–5
Normal Control #14	5/335 1.5% iii–2 v–3	5/413 1.2% iii–3 v–2	8/375 2.1% iii–4 v–4	11/376 2.9% iii–5 v–5 vi–1
Experimental (Infected) #9	35/290 12.1% iii–19 v–16	25/375 6.7% iii–14 v–11	37/357 10.4% iii–21 v–16	31/376 8.2% iii–15 v–16
Experimental (Infected) #11	45/290 15.5% ii–5 iii–20 v–20	36/310 11.6% ii–3 iii–14 v–19	43/405 10.6% iii–21 v–18 vi–4	29/354 8.2% iii–15 v–14
Experimental (Infected) #17	24/340 7.1% iii–13 v–11	60/305 19.7% ii–7 iii–28 v–20 vi–5	32/355 9.0% iii–16 v–16	39/378 10.3% iii–19 v–20
Experimental (Infected) #19	65/325 20.0% ii–5 iii–35 v–20 vi–5	55/365 15.1% ii–3 iii–23 v–23 vi–6	28/325 8.6% iii–15 v–13	35/297 11.8% iii–16 v–19

Control animals were not infected with FIV and the experimental (infected) animals were infected with FIV for 27 months. Data were analyzed with a two-factor ANOVA, the control (uninfected) and experimental (infected) were the between-subjects factor, and the anatomic location the repeated measures factor. The percentages of cells immunostained in the infected group was significantly greater than that for the uninfected group; F (1,4)=10.3, $p<0.01$. No differences due to the anatomic location were observed.

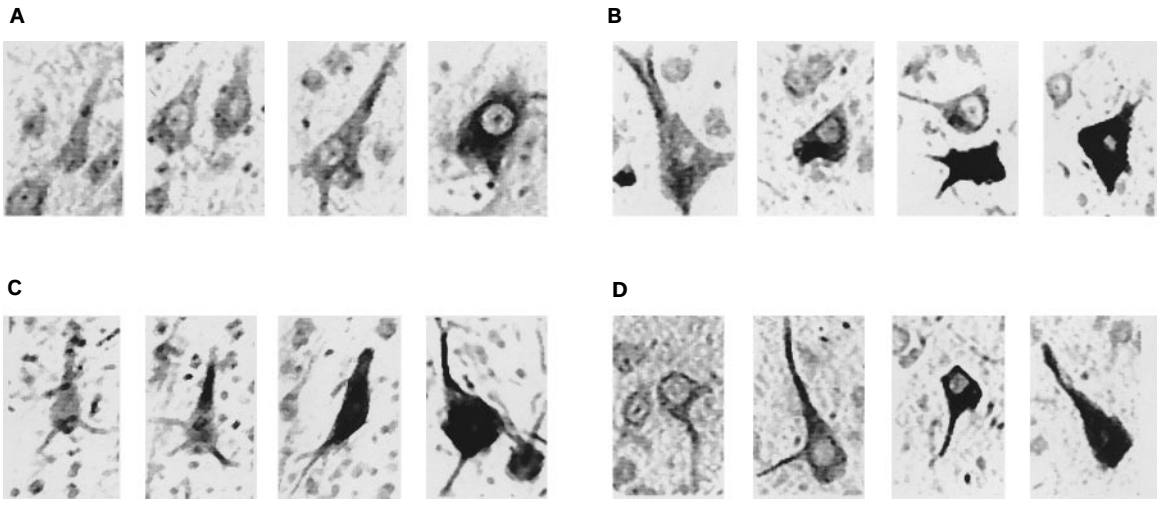

Figure 1 Digitized images of immunocytochemical staining with the SMI 32 antibody (original magnification 200×). These data were used in the image analysis techniques to obtain the quantitative data in Table 1. (a) Neurons in control cat #14, showing faint staining within the cell body and dendrites. (b) Neurons in cat #3, chronically implanted with cannulae. Numerous strongly stained cells were present. (c, d) Neurons in FIV-infected cats 17 and 19, respectively, showing a range of staining intensities with patchy as well as more intense staining.

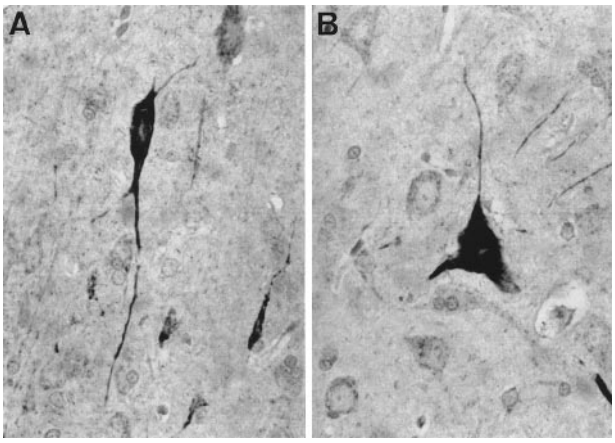

Figure 2 Photomicrographs of immunocytochemical staining with the SMI 32 antibody (original magnification 128×). (a, b) Positively stained neurons in FIV-infected cats #11 and 9, respectively, which could be found relatively frequently.

(Schlaepfer, 1987; Troncoso *et al*, 1985, 1986; Siegel *et al*, 1993; Sternberger and Sternberger, 1983; Trojanowski, 1991, 1993; Vickers *et al*, 1992). The findings of the present study are consistent with neurofilament changes due to FIV-induced neurological disease.

In the control group, we found staining for SMI 32 in 1.2–2.9% of the pyramidal neurons labeled in layers iii and v of the motor, premotor, prefrontal and anterior cingulate. We assume that this staining represents a normal dynamic process in the cytoskeleton. When we examined these same four regions in the brains of animals infected with FIV, we noted a significant increase (7–20%) in the number of cells stained (Table 1). We hypothesize that this significant increase in the labeling of the non-phosphorylated filaments in neuronal cells is a consequence of the inoculation with FIV, representing an abnormal increase in the neurofilaments, and is more than just normal replacement of the elements in the neuronal cytoskeleton.

The increased labeling of neurofilaments in the current study on the feline brain are similar to abnormalities in the neurofilaments reported by other investigators in neuronal tissue from neurological diseases in animals and humans. Spontaneously occurring and induced disease in animals demonstrate alterations in neurofilaments, as a result of hereditary canine spinal muscular atrophy (Cork *et al*, 1982, 1988), motor neuron disease in pigs (Higgins *et al*, 1983; Shields and Vandevelde, 1983), ataxia in horses (Montali *et al*, 1974), goats with copper deficiency (Schlaepfer, 1987), 'Shaker' calves (Rousseaux *et al*, 1985), social deprivation in monkeys (Siegel *et al*, 1993), and aluminium-induced pathology (Troncoso *et al*, 1986). In the human, there are also many examples of increased neurofilament staining and in progression to neurofibrillary tangles and neuritic plaque formation (Bouras *et al*, 1994; Hof *et al*, 1992; Lewis *et al*, 1987; Masliah *et al*, 1993 Price, 1994; Schlaepfer, 1987; Shields and Vandevelde, 1983; Sternberger and Sternberger, 1983; Trojanowski *et al*, 1991, 1993; Troncoso *et al*, 1986; Vickers *et al*, 1992).

Morrison and colleagues have also used the SMI 32 monoclonal antibody and noted changes in the non-phosphorylated proteins in patients with Alzheimer's disease (Bouras *et al*, 1994; Hof *et al*, 1992; Lewis *et al*, 1987; Vickers *et al*, 1992). They have proposed that the neurofilament proteins are vulnerable in neurodegenerative diseases including Alzheimer's dementia, Huntington's chorea, Parkinsonian disease and ALS. The effect is especially marked in the CA1 pyramidal cells of the dentate gyrus which show marked increases in non-phosphorylated neurofilament proteins (Trojanowski *et al*, 1993). In Alzheimer's disease and in the process of normal aging, there was increased staining of the neurofilaments in layer ii of entorhinal cortex while in the normally aging patient the prefrontal cortex was without any increase in fibrils (Vickers *et al*, 1992). In the Alzheimer's patient, the increase is noted in layer ii of the entorhinal cortex and throughout prefrontal cortexes.

In the current study in the cat, we noted staining in the control and FIV groups primarily in layers iii and v of the frontal lobe, with a significant increase found in the number of stained cells in the experimental group. Within FIV-infected subjects we found no significant difference in the labeling in motor, premotor, prefrontal or anterior cingulate areas (Table 1). The cells in layer ii and iii form callosal and association connections while those in layer v and vi form the subcortical projections to the corpus striatum, thalamus, brain stem and spine (Goldman and Nauta, 1977; Jones and Wise, 1977; Jones *et al*, 1979; Trojanowski and Jacobson, 1976).

Hof *et al* (1992) noted a difference in the laminar distribution of neurofibrillary tangles in the medial temporal lobe in patients with Alzheimer's or dementia pugilistica. In the Alzheimer's patients, the bulk of the labeled cells was in layer v while in the patients with dementia pugilistica, labeled cells were predominately found in layers ii–iii. In our study of granular and agranular motor cortex, association cortex and anterior cingulate regions, we found that the basic pattern was similar in that pyramidal cells in layers iii and v were primarily labeled.

Our findings in the frontal lobe of the cat are the first demonstration in an animal model for AIDS of changes in the neuronal cytoskeleton in a region which is responsible for motor function and motivation. Since SMI 32 immunoreactivity has not been well characterized in the cat, we also used this antibody to examine neuronal staining patterns in the cortex of a cat with cannulae implanted bilateraly through the lateral cerebral gyrus into the

lateral ventricle for 7 months. In this animal with chronic injury to the brain, an increased percentage of pyramidal neurons was seen near the cannula tract with staining qualities similar to those seen in the FIV-infected cats (Figure 1B). This suggests that enhanced SMI 32 reactively in the feline brain can occur in response to an insult; in the case of our experimental cats, infection with FIV.

One of the major sequelae in humans with HIV-1 associated encephalitis is cerebral atrophy. Although in one evaluation of brain tissue from humans with HIV-1 encephalitis, no actual decrease in the number of neurons was found (Seilhean *et al*, 1993), the majority of studies do report a generalized or localized reduction in numbers of neurons (Ketzler *et al*, 1990; Oster *et al*, 1995; Weis *et al*, 1993; Wiley *et al*, 1991). The studies that have shown neuronal loss have also shown a reduction of dendritic processes and a decrease in synaptic density (Masliah *et al*, 1992a,b; Price *et al*, 1988; Price, 1994; Weiss *et al*, 1993; Wiley *et al*, 1991). We do note a trend of a decreased total number of neurons in the infected cats, particularly in premotor area 6, but we are limited by our sample size and counting method, as more quantitative stereological techniques are required to confirm neuronal loss. Future studies will address the relationship between increased SMI 32 reactivity and potential neuronal drop-out in FIV-infected cats.

Using the SMI 32 monoclonal antibody, we demonstrate a significant increase in the labeling of neurofilaments in the pyramidal neurons in the frontal lobe of the FIV-infected cats. It has been noted in other studies that an increase in the number of neurofilaments may lead to several pathological conditions including interference with axonal transport of neurofilaments, formation of neurofibrillary tangles, or neuronal atrophy (Cork *et al*, 1988; Masliah *et al*, 1992a; Price, 1994; Schlaepfer, 1987; Shields *et al*, 1983; Trojanowski *et al*, 1991; Troncoso, 1985). In the current study on the FIV-infected cats, we cannot determine the functional consequences from this increase in non-phosphorylated neurofilaments, although several neurological abnormalities were found in these cats. However, the enhanced staining we noted does suggest that these pyramidal cells represent a vulnerable population.

The SMI 32 monoclonal antibody reacted only with pyramidal cells in the cortex, and we never noted staining of granule cells. Other methods will be needed to determine if the granule cells, which form much of the local circuits in the brain, are also affected by this disease process. Our findings indicate that this method can be used to identify changes occurring in the pyramidal cells in the frontal lobe of infected animals. We believe these findings validate the importance of the FIV/cat model. Future studies will undertake therapeutic intervention to alleviate the consequences of the FIV infection on neurological parameters and examination of the effects on the neuronal cytoskeleton will serve as a valuable readout for a measure of success of such treatments.

Materials and methods

Animals

Six specific-pathogen free, 2 month old, female cats were obtained from Liberty Laboratories, and two animals were placed in the control group and four animals in the treatment group. Animals were housed communally within a treatment group and each group was housed in different rooms. This method of housing fosters animal interactions and helps to create a mentally stimulating environment without the potential of cross-transmission of virus to control animals. Another cat from a previous study with bilaterally implanted cannulae in the lateral ventricles served as a control to determine the staining quality of the SMI 32 antibody in chronic brain injury.

Virus inoculation

FIV-MD was employed in these studies (Phillips *et al*, 1994). One cat, #11, was inoculated with 2 ml of heparinized blood from another cat previously infected with this virus, whereas three cats (#9, 17 and 19) were inoculated with the virus from tissue culture supernatant. Seroconversion rate and disease course in cat #11 was similar to those observed in the other infected animals. Animals were infected at 10 weeks of age and all animals were 27 months post-infection at time of sacrifice. At this stage, animals were all seropositive for the virus, had neurological abnormalities in evoked potentials and sleep parameters (Phillips *et al*, 1994), but were free from opportunistic infection and severe immunodeficiency. FIV could be isolated from the terminal CSF samples from two of the four infected cats (#17 and 19).

Histopathology

At termination, each cat was injected with an overdose of ketamine hydrochloride/Rompine and perfused with 2 liters of phosphate buffered saline at pH 7.4 containing 1 U heparin/ml to clear the blood cells from the brain. The brains were rapidly removed and divided for tissue culture and pathology. The portion prepared for pathology was immersion fixed in 10% neutral buffered formalin within 15 min of death. The brain was sliced into 12 thick coronal sections and post-fixed in neutral buffered formalin for 24 h, transferred into 70% ethanol for 16 h, and processed for paraffin embedding.

Six micron sections from each block were stained with hematoxylin and eosin (H&E) for routine histopathological analysis. Indirect immunocytochemical analysis was performed on deparaffinized

sections using the avidin-biotin complex/horseradish peroxidase technique. Color was developed with the VIP reagent (Vector Labs) and slides were lightly counterstained with Gills' hematoxylin. Antibodies utilized were MAC387 (DAKO) to identify infiltrating or activated macrophages (used at 1/300 dilution and for which sections were pretreated with 0.1% trypsin), and Sternberger Monoclonal International (SMI) monoclonal antibody 32 (used at 1/1000 dilution and for which sections were pretreated with microwave irradiation in citrate buffer, pH 6.0). One section was immunocytochemically stained with each antibody, a negative control lacking primarily antibody was also performed. The antibody SMI 32 recognizes a non-phosphorylated epitope in neurofilament H which is masked when the epitope is phosphorylated (Sternberger and Sternberger, 1983).

Image analysis
All sections were examined blind. The tissue was analyzed with an image analysis system consisting of a Zeiss Axiophot microscope with a color Hitachi TV camera. The sections were examined at a magnification of 200×, and the images collected with a grabber program, Snap It, developed at TSRI by Dr Warren Young. The images were stored on a Fujitsu optical disc. The sections were later analyzed with the aid of NIH Image 1.56 and number of cells per cortical area, number of cells per layer, and the surface area of the labeled and unlabeled cells were recorded. Quantitative counts were performed in triplicate.

The cortical analysis was to areas defined by Harlow and Woolsey (1965) and Winkler and Potter (1914). In this analysis, we examined the following cortical regions: motor (agranular motor – area 4), premotor (granular motor – area 6), prefrontal (proreus, granular association – area 12) and anterior cingulate cortexes (homotypical – area 32). The four areas were identified and a vertical column 1 mm in width reaching from layers, i–vi was selected for study in each animal. The total number of neurons in the section stained by the hematoxylin counterstain was determined for each column of cells.

The positively labeled cells with the SMI 32 were identified as to cortical area, layer and the percent of labeled cells in the cortex (Table 1). Cells showing filamentous staining in the cell body were considered positive with the SMI 32 monoclonal antibody (Figure 2). Additionally dendrites were frequently immunolabeled. With the SMI 32 antibody we noted labeling of only pyramidal cells.

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References

Bershadsky AD, Vasiliev JM (1988). *Cytoskeleton*. Plenum Press: New York.

Boche D, Hurtrel M, Gray F, Maire MA, Ganiere JP, Montagnier L, Hurtrel B (1996). Virus load and neuropathology in the FIV model. *J Neurovirol* **2:** 377–387.

Bouras C, Hof PR, Giannakopoulos P, Michel J-P, Morrison JH (1994). Regional distribution of neurofibrillary tangle and senile plaques in the cerebral cortex of elderly patients: a quantitative evaluation of a 1 year autopsy population from a geriatric hospital. *Cerebral Cortex* **4:** 138–150.

Cork LC, Griffin JW, Choy C, Padula CA, Price DL (1982). Pathology of motor neurons in accelerated hereditary canine spinal muscular dystrophy. *Lab Invest* **46:** 89–99.

Cork LC, Troncorso JC, Klavano GG, Johnson ES, Sternberger LA, Sternberger NH, Price DL (1988). Neurofilamentous abnormalities in motor neurons in spontaneously occurring animal disorders. *J Neuropath Exp Neuro* **47:** 420–435.

Goldman PS, Nauta WJH (1977). Columnar distribution of corticocortical fibers in the frontal association limbic and motor cortex of the developing rhesus monkey. *Brain Res* **122:** 393–415.

Harlow HJF, Woolsey CN (1965). *Biological and Biochemical Basis of Behavior*. Univ. of Wisconsin Press: Madison, Wis, pp 63–152.

Henriksen SJ, Prospero-Garcia O, Phillips TR, Fox HS, Bloom FE, Elder JH (1995). Feline immunodeficiency virus as a model for study of lentivirus infection of the central nervous system. In: *Current Topics in Microbiology and Immunology: HIV and Dementia*. Oldstone MBA, Vitkovic L, (eds). **202:** 167–186.

Higgins RJ, Rings DM, Fener WR, Stevenson S (1983). Spontaneous lower motor neuron disease with neurofibrillar accumulations in young pigs. *Acta Neuropath Berl* **59:** 288–294.

Hof PR, Bouras C, Buee L, Delacourte A, Perl DP, Morrison JH (1992). Differential distribution of neurofibrillar tangles in the cerebral cortex of dementia pugilistica and Alzheimer's disease cases. *Acta Neuropath* **85:** 23–30.

Hurtrel M, Ganiere JP, Guelfi JF, Chakrabarti L, Maire MA, Gray F, Montagnier L, Hurtrel B (1992). Comparison of early and late FIV encephalopathies. *AIDS* **6:** 399–406.

Jones EG, Wise SP (1977). Size and laminar columnar distribution of efferent cells in the sensory-motor cortex of monkeys. *J Comp Neurol* **175:** 391–438.

Lafrado L, Podell M, Krakowa S, Hayes K, Hanlon M, Mathes L (1993). FIV: a model for retrovirus-induced pathologenesis. *AIDS Res Rev* **3:** 115–150.

Jones EG, Coulter JD, Wise SP (1979). Commissural columns in the sensory motor cortex of monkeys. *J Comp Neurol* **188:** 113.

Ketzler S, Weisl S, Haug H, Budka H (1990). Loss of neurons in the frontal cortex in AIDS brains. *Acta Neuropathol* **80:** 92–94.

Lewis DA, Campbell MJ, Terry RD, Morrison JH (1987). Laminar and regional distributions of neurofibrillar tangles and neuritic plaques in Alzheimer's disease: a quantitative study of visual and auditory cortex. *J Neurosci* **7:** 1799–1808.

Masliah E, Ge N, Morey M (1992a). Cortical dendritic pathology in HIV encephalitis. *Lab Invest* **66:** 285–291.

Masliah E, Achim CL, Ge N, DeTeresa R, Terry RD, Wiley CA (1992b). Spectrum of human immunodeficiency virus-associated neocortical damage. *Ann Neurol* **32:** 321–329.

Masliah E, Mallory M, Hansen L, Alford M, deTeresa R, Terry RD (1993). An antibody against phosphorylated neurofilaments identifies a subset of damaged association axons in Alzheimer's disease. *Am J Pathol* **142:** 871–882,

Mitchison T, Kirschner M (1988). Cytoskeletal dynamics and nerve growth. *Neuron* **1:** 761–772.

Montali RJ, Bush M, Sauer RM, Gray CW, Xanten WA (1974). Spinal ataxia in zebras. Comparison with the wobbler syndrome of horses. *Vet Pathol* **11:** 68–78.

Oster S, Cristoffersen P, Gundersen HJ, Nielsen JO, Pedersen C, Pakkenberg B (1995). Six billion neurons lost in AIDS. A stereological study of the neocortex. *APMIS* **102:** 525–529.

Phillips TR, Prospero-Garcia O, Puaoi DL, Lerner DL, Fox HS, Olmsted RE, Bloom FE, Henriksen SJ, Elder JH (1994). Neurological abnormalities associated with feline immunodeficiency virus infection. *J Gen Virol* **75:** 979–987.

Phillips TR, Prospero-Garcia O, Wheeler DW, Wagaman PC, Lerner DL, Fox HS, Whalen LR, Bloom FE, Elder JH, Henriksen SJ (1996). Neurologic Dysfunctions Caused by a Molecular Clone of Feline Immunodeficiency. *J Neurovirol* **2:** 388–396.

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, Cleary P (1988). The brain in AIDS: central nervous system HIV-1 infection and AIDS Dementia Complex. *Science* **239:** 586–592.

Price RW (1994). Understanding the AIDS Dementia complex. The challenge of HIV and its effects on the central nervous system. In: *HIV, AIDS and the Brain*. Price RW, Perry SW, (eds). Raven Press: New York, pp 1–45.

Rousseaux CG, Kalano GG, Johnson ES, Shnitkae TK, Harriee WN, Snyder FF (1985). 'Shaker' calves syndrome a newly recognized inheritive neurodegenerative disease of horned Hereford calves. *Vet Pathol* **22:** 104–112.

Schlaepfer WW (1987). Neurofilaments, structure, metabolism, and implications in disease. *J Neuropath Exp Neurol* **46:** 117–129.

Seilhaen D, Duyckaerts C, Vazeus R, Bolgert P, Brunet P, Katlama C, Gentilin M, Hauw JJ (1993). HIV-1 associated cognitive/motor complex: Absence of neuronal loss in the cerebral neocortex. *Neurology* **43:** 1492–1499.

Shields RP, Vandevelde M (1983). Spontaneous lower motor neurons disease with neurofibrillary accumulations in young pigs. *Acta Neuropath Berl* **59:** 288–294.

Siegel SJ, Ginsberg SD, Hof PR, Foote SL, Young WG, Kraemer GW, McKinney WT, Morrison JH (1993). Effects of social deprivation in prepubescent rhesus monkeys: Immunohistochemical analysis of the neurofilament protein triplet in the hippocampal formation. *Brain Res* **619:** 299–305.

Sternberger LA, Sternberger NH (1983). Monoclonal antibodies distinguish phosphorylated and nonphosphorylated form of neurofilaments in situ. *Proc Natl Acad Sci USA* **80:** 6126–6130.

Trojanowski JQ, Jacobson S (1976). The areal and laminar distribution of pulvinar cortical afferents in rhesus monkeys. *J Comp Neurol* **169:** 371–392.

Trojanowski JQ, Schmidt ML, Otvos L, Arai H, Hill WD, Lee VM-Y (1991). Vulnerability of the neuronal cytoskeleton in Alzheimer's disease; widespread involvement of all three major filament systems. *Ann Rev Gerontol Geriatr* **10:** 167–182.

Trojanowski JQ, Schmidt ML, Shin R-W, Bramblett GT, Rao D, Lee VM-Y (1993). Altered tau and neurofilament proteins in neurodegenerative diseases. Diagnostic implication for Alzheimer's diseases and Lewy body dementia. *Brain Pathol* **3:** 45–54.

Troncoso JC, Hoffmani PN, Griffin JW, Hess-Kozlow KM, Price DL (1985). Aluminium intoxication; a disorder of neurofilament transport in motor neurons. *Brain Res* **342:** 172–175.

Troncoso JC, Sternberger NH, Sternberger LA, Hoffman PN, Price DL (1986). Immunocytochemical studies of neurofilament antigens in the neurofibrillar pathology induced by aluminium. *Brain Res* **364:** 295–300.

Vickers JC, Delacourtae A, Morrison JH (1992). Progressive transformation of the cytoskeleton associated with normal aging and Alzheimer disease. *Brain Res* **594:** 273–278.

Weis S, Haug H, Budka H (1993). Neuronal damage in the cerebral cortex of AIDS brains: a morphometric study. *Acta Neuropathol* **85:** 185–189.

Wiley CA, Masliah E, Morey M, Lemere C, De Teresa R, Grafe M, Hansen L, Terry R (1991). Neuronal damage during HIV infection. *Ann Neurology* **29:** 651–657.

Winkler D, Potter A (1914). *An Anatomical Guide to Experimental Researches on the Cat's Brain*. W Versluys: Amsterdam.