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Neurologic dysfunctions caused by a molecular clone of Feline Immunodeficiency virus, FIV-PPR

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FIV is a lentivirus of domestic cats that causes a spectrum of diseases that is remarkably similar to the clinical syndrome produced by HIV infection in people. Both HIV and FIV has been shown to cause neurologic dysfunction. Specific Pathogen-Free (SPF) cats were placed into one of three groups: FIV-PPR infected; DU-FIV-PPR (a dUTPase mutant of the FIV-PPR clone) infected; or an age-matched control group. In both infected groups, the general clinical signs of infection included lymphadenopathy, oral ulcerations, rough hair coat, and conjuntivitis. Specific neurological changes in the FIV-PPR infected cats included hind limb paresis; delayed righting and pupillary reflexes; behavioral changes; delayed visual and auditory evoked potentials; decreased spinal and peripheral nerve conduction velocities; and marked alterations in sleep patterns. Most of these changes were also observed in the DU-FIV-PPR infected cats. However, these cats tended to have a slightly less severe disease. In this study, we have demonstrated that an infectious molecular clone of FIV closely parallels the disease course of wild type FIV-infected cats. By using a knockout gene mutant of this clone, we were able to demonstrate that the dUTPase gene is not essential for neuropathogenesis. Further use of the FIV-PPR clone should prove useful in determining the essential viral elements that are important in the neuropathogenesis of lentiviral infections.

Keywords: FIV; neurologic disease; HIV; lentivirus; electrophysiology; dUTPase

Introduction

Both human immunodeficiency virus (HIV-1) and feline immunodeficiency virus (FIV) are lentiviruses and share many common biochemical and structural properties (reviewed in Elder and Phillips, 1994). Additionally, the disease that each of these viruses produces in their respective host is very similar, with both viruses causing immunosuppression and neurological dysfunction (Pedersen *et al.*, 1987; Egberink *et al.*, 1988; Elder and Sever, 1988; Dow *et al.*, 1990; Davis *et al.*, 1992; Hurtrel *et al.*, 1992; Wheeler *et al.*, 1992; Podell *et al.*, 1993; Henriksen *et al.*, 1994). Because of these similarities, FIV infection in the cat represents a good model for examining lentiviral pathogenesis as well as potential therapeutic interventions. The primary interest of our laboratories has been to develop FIV as a model for the study of the neurological aspects of the disease. In order to determine, at the molecular level, which viral properties are important for the induction of CNS dysfunction, it is essential to develop a neuropathogenic molecular clone.

FIV-PPR is an infectious molecular clone that has been completely sequenced and extensively characterized at the molecular level (Phillips *et al.*, 1990, 1992; Elder *et al.*, 1992, 1993; Wagaman *et al.*, 1993; Mancuso *et al.*, 1994; Prospero-Garcia *et al.*, 1994). Because of this extensive characterization of FIV-PPR, demonstrating neurovirulence for this clone would make it a valuable tool in determining the *in vivo* relevance of various genetic manipulations made to the virus.

The purpose of this study was twofold: (1) to determine whether the infectious molecular clone, FIV-PPR, is neurovirulent, and (2) to determine whether the DU gene plays a vital role in the neuropathogenesis of FIV.

The DU gene of FIV encodes an enzyme with dUTPase activity (reviewed in Elder and Phillips,

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1994). Although this enzyme is not encoded by the primate lentiviruses, most of the non-primate lentiviruses contain the DU gene (Elder and Phillips, 1994). It has been demonstrated *in vitro* that a knockout mutation of DU reduces macrophage replication efficiency of both FIV and equine infectious anemia virus (Threadgill *et al.*, 1993; Wagaman *et al.*, 1993). Since macrophages are thought to be an important mode of lentivirus entry in the brain, we examined the potential role that DU may play in FIV neurovirulence.

The results of this study demonstrate that FIV-PPR is a neurovirulent molecular clone and that mutational inactivation of the DU gene of this clone does not substantially alter its neurovirulence.

Results

All of the infected cats developed an FIV antibody response by 8 weeks post-infection, with no detectable difference occurring in the level or kinetics of the antibody response (Lerner *et al.*, 1995). At 16 weeks post-infection, virus was isolated from all of the FIV-PPR and DU-FIV-PPR infected cats. From PCR amplification and nucleotide sequence analysis, it was determined that all the DU-FIV-PPR inoculated cats maintained the inserted DU mutation (data not shown).

The primary focus of this study was to examine the neurologic aspects of the FIV induced disease. Clinical observations between the two groups of infected cats were similar during the course of this study. Both FIV-PPR and DU-FIV-PPR infected animals demonstrated readily observable clinical abnormalities. Disease signs are summarized in Table 1. Particularly relevant to this study were the neurologic abnormalities (delayed pupillary and righting reflexes, behavioral changes, and hind limb paresis). The posterior paresis was manifested by an uncoordinated hind limb gait and the inability to successfully negotiate a 6 inch jump. As we have previously reported with the Maryland strain of FIV (FIV/MA), one of the cats changed its behavior from a people-oriented animal to a cat with a more solitary behavior and greater aggression (Phillips *et al.*, 1992). By 7 months post-infection, all of the clinically detectable neurologic abnormalities had dissipated.

Table 1 Observed clinical abnormalities

Clinical signs	*PPR cats*	*DU (–) cats*	*Onset post-infection*
Lymphadenopathy	++++	++++	5 weeks
Rough hair coat	+++	++	7 weeks
Delayed righting reflex	++	+	9 weeks
Oral ulcers	+	++	9 weeks
Hind limb paresis	++		9 weeks
Delayed pupillary reflex (direct and consensual)	+	+	12 weeks
Behavioural changes	+		14 weeks
Conjunctivitis	+	+	20 weeks

To better define the clinical neurological abnormalities and possible presence of subclinical neurological abnormalities, electrophysiologic assessment of the spinal cord and peripheral nerves was initiated. Electrophysiological abnormalities were found in all infected cats (Tables 2 and 3). The CNS had more abnormalities than the PNS. Central conduction velocities were delayed greater than two standard deviations below the control mean in all four of the PPR-FIV infected cats, and in three of the four DU-FIV-PPR infected cats (Table 2). The cervical region was most severely affected in the FIV-PPR cats, followed by the lumbar region. In the DU cats, the lumbar region showed the greatest number of abnormalities. Delayed motor and sensory peripheral conduction velocities were found in just one FIV-PPR cat, while the sensory conduction velocity was delayed in two DU cats (Table 2). Comparison of the conduction velocities at 5 months post-infection and 19 months post-infection revealed no obvious pattern to the abnormalities. Some of the cats increased their conduction velocities, some remained stable, and some velocities decreased. In some cats the velocities did all three depending upon which portion of the nervous system was being examined. For example, in cat #30 (FIV-PPR), the cervical spinal cord conduction velocity was significantly delayed at 5 months post-infection and more so at 19 months post-infection. Whereas, thoracic and lumbar conduction velocities improved from significantly delayed at 5 months post-infection to normal values at 19 months post-infection. Only cat #25 (DU-FIV-PPR) had no abnormal values for conduction velocities at either recording session. However, if the duration of peripheral sensory evoked potentials are examined, then cat #25 must also be considered as having abnormal recordings (Table 3). Similar findings (normal peripheral sensory conduction velocities with increased evoked potential duration) were also seen in two FIV-PPR infected cats and two other DU-FIV-PPR infected cats.

Further neurologic assessment was performed by conducting visual and auditory evoked responses. Both of these assays were recorded at 3 and 11 months post-infection. At 3 months post-infection, examination of the visual evoked potentials revealed that the latency of the P3 wave was significantly greater in both the FIV-PPR and the DU-FIV-PPR infected cats relative to the controls (Figure 1), but there were no significant differences between FIV-PPR and DU-FIV-PPR. In three of the four FIV-PPR infected cats, the latency value for the P3 wave increased at 11 months post-infection relative to the value recorded at 3 months post-infection. The average P3 latency of the control cats

Table 2 Spinal cord and peripheral nerve conduction velocities (m/s) of FIV-PPR and DU-FIV-PPR infected cats compared to control cats

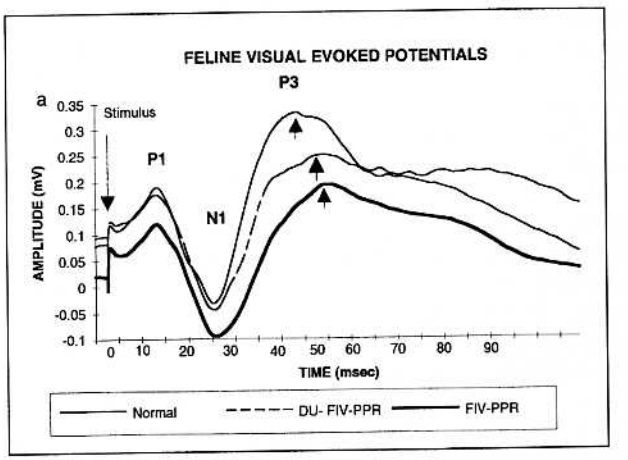
	Cervical		*Thoracic*		*Lumbar*		*Motor*		*Sensory*	
PPR cat no.	*5 mo*	*19 mo*	*5 mo*	*19 mo*	*5 mo*	*19 mo*	*5 mo*	*19 mo*	*5 mo*	*19 mo*[d]
23	**53**[a]	**112**[b]	86	93	57	65	74	63	80	75
24	99	ND	82	ND	**55**[a]	ND	69	ND	80	ND
26	**94**[a]	**115**[b]	82	86	**50**[a]	**68**[b]	67	79	72	77
30	**91**[a]	**78**[a]	**71**[a]	85	**53**[a]	**56**[a]	93	**59**[a,b]	**59**[a]	**85**[b]
DU-Cat no.										
25	101	113	88	93	57	67	64	56	89	76
27	97	109	82	81	**50**[a]	**81**[b]	63	69	**71**[a]	73
28	**93**[a]	ND	**76**[a]	ND	58	ND	66	ND	**68**[a]	ND
29	96	**118**[b]	85	90	**55**[a]	59	79	75	78	80
Control Ave[c]	104.14		85.14		61.00		70.71		79.83	
±s.d.	±4.34		±2.61		±2.97		±4.39		±4.36	

[a] greater than two standard deviations below the control average
[b] no. greater than 20% between recording of individual animals
[c] $n=7$ for this assay historical age matched controls were available and added
[d] time post-infection
ND=not done

Table 3 Duration factor[b] for the peripheral sensory evoked potentials (ms) of FIV-PPR and DU-FIV-PPR infected cats compared to control cats

PPR Cat no.	*5 months*	*19 months*
23	16	16
24	**19**[a]	ND
26	**20**[a]	**22**[a]
30	16	16
DU-Cats		
25	**19**[a]	**32**[a]
27	**18**[a]	**18**[a]
28	17	ND
29	**18**[a]	16
Control Ave.[c]	14.85	
±s.d.	±1.46	

[a] greater than two standard deviations above the control average
[b] The Duration Factor is derived by dividing the duration of the potential (in msec) by the length of the nerve conducting the potential (in meters). This is done to compensate for the variation in the size of the cats
[c] $n=7$, for this assay historical age matched controls were available and added
ND=not done



b

Groups	P1	N1	P3
Normal	11.63	25.85	45.36
n=5	±0.01	±0.2	±1.23
PPR-dU	11.18	24.58	**53.18***
n=4	±0.01	±0.78	±2.47
PPR	11.53	25.62	**55.18***
n=4	±0.17	±1.41	±0.6

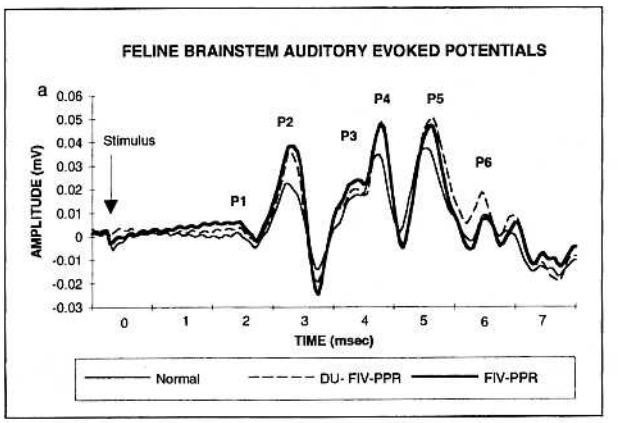
Latencies in msec. *p<0.01

Figure 1 Comparison of the VEPs superaverage. (a) shows the superaverage from Control, FIV-PPR infected, and DU-FIV-PPR infected cats. (b) shows the latency of the different waves. P3 was significantly delayed in the FIV-PPR infected and DU-FIV-PPR infected cats.

was 45.36 ms at both 3 and 11 months post-infection. However, the P3 latency for cat #24, for example, at 3 months post-infection, was 56.80 ms and increased to 57.85 ms when recorded at 11 months post-infection.

For the auditory evoked potentials, both the FIV-PPR and the DU-FIV-PPR infected cats had a significantly increased mean P5 latency compared to the control values (Figure 2). At 11 months post-infection, this alteration in the auditory evoked potential persisted, but did not progress (data not shown).

One cat from each of the groups (control, FIV-PPR, and DU-FIV-PPR) was surgically implanted with permanent nichrome screw electrodes to



b

Groups	P1	P2	P3	P4	P5	P6
Normal	1.95	2.84	3.94	4.32	5.04	6.08
n=5	±0.04	±0.05	±0.03	±0.04	±0.03	±0.04
PPR-dU	1.99	2.89	3.81	4.35	**5.20***	6.06
n=4	±0.05	±0.06	±0.23	±0.02	±0.03	±0.01
PPR	1.96	2.75	3.75	4.32	**5.19***	6.08
n=4	±0.1	±0.0	±0.04	±0.01	±0.02	±0.01

Latencies in msec. *p<0.04

Figure 2 Comparison of the superaverage BAEPs. (a) Illustrates the superaverage traces obtained from the Control, FIV-PPR infected and DU-FIV-PPR infected groups. (b) Contains the numerical values for the latencies of the indicated waves. The latency of the P5 wave of the FIV-PPR infected and DU-FIV-PPR infected cats exhibits a significant delay relative to the values obtained from the control cats.

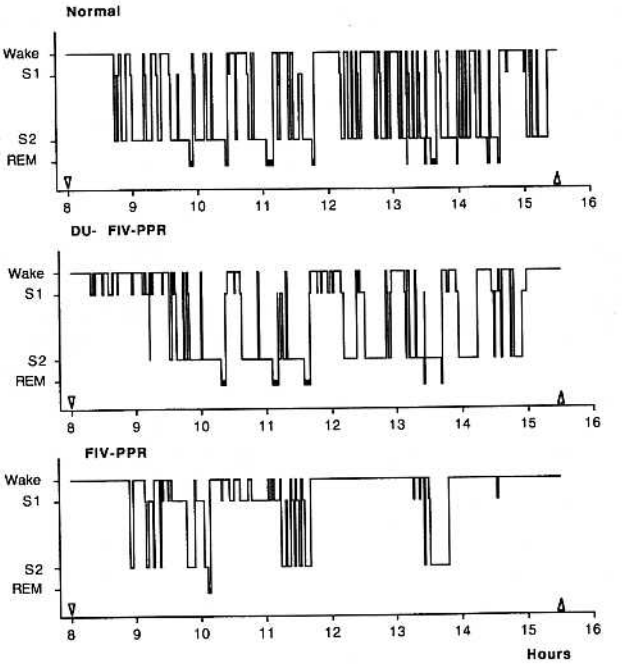


Figure 3 Effects of FIV-PPR and DU-FIV-PPR on sleep architecture. Each of the above hypnograms represent the sleep recording of a cat from the Control, FIV-PPR infected, or DU-FIV-PPR infected groups. In FIV-PPR infected and DU-FIV-PPR cats, the latency for SWS2 sleep onset was substantially increased, total amount of sleep time was decreased, and amount of rapid eye movement (REM) sleep was decreased, when compared to the control animal.

assess sleep-wake changes. Analysis of 8 h sleep recordings in the three subjects revealed dramatic changes in sleep parameters in the FIV-infected cats compared to the control. Figure 3 illustrates the changes in the sleep hypnogram in the FIV-PPR and DU-FIV-PPR infected cats relative to the control. Striking alterations in the hypnogram are demonstrated in both of the FIV infected cats. There was an increased latency in the onset of SWS2 sleep, a loss in the total amount of REM sleep, and a decreased amount of total sleep. These changes in the sleep architecture in the FIV-PPR and DU-FIV-PPR infected cats were similar to the changes that we have previously reported in FIV/MA infected cats (Phillips *et al.*, 1994; Prospero-Garcia *et al.*, 1994) as well as changes reported for HIV-infected humans (Aldrich *et al.*, 1988; Norman *et al.*, 1988, 1990, 1992; St Kubicki *et al.*, 1988; Rothenberg *et al.*, 1990).

Discussion

In this study, we have demonstrated that the infectious molecular clone of FIV, FIV-PPR, causes a disease which is similar to the disease caused by uncloned strains of the virus (Wheeler *et al.*, 1992; Podell *et al.*, 1993; Phillips *et al.*, 1994). FIV-PPR and DU-FIV-PPR each produced the general disease signs of lymphadenopathy, oral ulcerations, rough hair coat, and conjuntivitis. Specific neurological alterations of delayed righting and pupillary reflexes, delayed visual evoked potentials, delayed auditory evoked potential, decreased spinal and peripheral nerve conduction velocities, qualitative abnormalities in the configurations of peripheral sensory evoked potentials, and marked alterations in sleep patterns were seen in both groups of infected cats. However, behavioral changes and hind limb paresis were only present in the FIV-PPR infected cats. Although DU-FIV-PPR infected cats clearly demonstrated neurological dysfunction, these animals tended to have a less severe disease than the FIV-PPR infected cats. This mild attenuation effect was seen in the clinical signs, visual evoked potentials, and sleep recording. It has been previously demonstrated *in vitro* that DU-FIV-PPR has a markedly reduced replication rate in macrophages relative to FIV-PPR (Wagaman *et al.*, 1993). Since the target cells for lentiviruses in the CNS are predominately macrophages and microglia, it was somewhat surprising that a DU minus mutant of FIV was still able to cause a neurological form of the disease that was only mildly attenuated. However, these results were not totally unexpected, as neither HIV-1 or SIV encode or contain dUTPase

activity and both of these viruses are known as neurologic disease producing agents (Elder and Phillips, 1994). Thus, from these studies we can conclude that FIV is similar to both HIV-1 and SIV infections, in that the DU gene is not essential for the development of the neurological form of the disease.

It is interesting to note that the behaviorally assessed neurological abnormalities were no longer detectable past 7 months post-infection. However, the brainstem and cortical electrophysiological abnormalities persisted, or progressed in the case of the visual evoked potentials. This indicates that while the brain was able to compensate for the initial viral insult, the neuropathogenic process still continued. Thus, it is likely that additional clinical neurological dysfunction will be seen in the future.

The pathophysiology of FIV on the spinal and peripheral nervous system differed from our finding in the brain. The data from the spinal and peripheral evoked potentials indicated no viral preference for a particular site within the feline spinal cord or peripheral nervous system, nor was the disease relentlessly progressive. Instead, it appeared that the affected portions of the nervous system, in many cases, were able to compensate for the viral insult and return to normal function. However, concomitantly, another region of the nervous system often demonstrated a diminished ability to conduct nervous impulses. This disease pattern produced by FIV may be the result of demyelination with a subsequent remyelination.

Although FIV-PPR is a molecular clone, the neurologic disease produced by this clone was very similar to the disease produced by FIV/MA, an uncloned viral swarm (Podell *et al.*, 1993; Phillips *et al.*, 1994; Prospero-Garcia *et al.*, 1994). Both FIV-PPR and FIV-MA produce readily observable neurologic disturbances early during the course of disease, as soon as 3 months post-inoculation (e.g. abnormal reflexes, increased latencies in both auditory and visual evoked potentials, decreased nerve conduction velocities, as well as changes in the normal sleep architecture) (Podell *et al.*, 1993; Phillips *et al.*, 1994; Prospero-Garcia *et al.*, 1994). The neurological dysfunctions in these FIV infected cats are similar to those found in HIV infected patients (Aldrich *et al.*, 1988; Norman *et al.*, 1988, 1990, 1992; St Kubicki *et al.*, 1988; Smith *et al.*, 1988; Malessa *et al.*, 1989; Goodin *et al.*, 1990; Koralnik *et al.*, 1990; Rothenberg *et al.*, 1990; Davis *et al.*, 1992; Pagano *et al.*, 1992; Boccellari *et al.*, 1993; Jabbarii *et al.*, 1993). Although both of these FIV strains produce similar systemic and neurologic disease, there are strain differences. A high percentage of our cats infected with FIV-MA developed anisocoria (Phillips *et al.*, 1994), while hind limb paresis was a common sequelae to FIV-PPR infection.

The results of these studies indicate that FIV is a good model for examining the early neurologic effects of lentiviruses. We have demonstrated that an infectious molecular clone of FIV can produce neurologic disease in cats that mimics the disease course of HIV infected people. This clone should prove useful in elucidating which lentiviral elements are important for neurovirulence.

Materials and methods

Viruses

FIV-PPR is an infectious molecular clone that has been completely sequenced and characterized at the molecular level (Phillips *et al.*, 1990, 1992; Elder *et al.*, 1992, 1993; Wagaman *et al.*, 1993; Mancuso *et al.*, 1994; Prospero-Garcia *et al.*, 1994). A mutation was placed into the DU gene of FIV-PPR, as previously described (Wagaman *et al.*, 1993). Briefly, an oligo, containing a SphI site, was inserted into the unique EcoRV site of the DU gene. This insertion destroyed DU activity but maintained the open reading frame, allowing normal integrase function (Wagaman *et al.*, 1993). This FIV-PPR mutant virus was designated DU-FIV-PPR. Infectious virus stocks for both clones were prepared by separately transfecting CrFK cells and co-cultivating with SPF-PBMCs, as previously described (Phillips *et al.*, 1990). The tissue culture infectious dose 50 ($TCID_{50}$) was determined by serial dilution of each virus in SPF-PBMCs and monitoring the dilutions for reverse transcriptase (RT) activity. The $TCID_{50}$ was then calculated by determining the dilution where 50% of the cultures had RT activity (Reed and Muench, 1938).

Animals

Specific pathogen free (SPF) cats were obtained from Liberty Laboratories (Liberty Cornersm NJ). The virus inoculation procedure was conducted, as previously described (Wagaman *et al.*, 1993; Phillips *et al.*, 1994). Cats were placed into three groups. Group 1 consisted of five cats that served as an age matched control group. Group 2 contained four animals which were intravenously inoculated with 300 $TCID_{50}$ units of DU-FIV-PPR, while Group 3 consisted of four animals which were intravenously inoculated with 300 $TCID_{50}$ units of FIV-PPR. At the time of inoculation, the cats were 10 weeks old. Each group of cats was communally housed, but separated from the other treatment groups.

Physical and neurologic examinations

Routine physical and neurologic exams were performed approximately once a month for the duration of the studies. The neurologic exam consisted of an overall assessment of behavior, gait and posture. Palpebral, menace, pupillary, righting, and patellar reflexes were examined. Hopping and wheelbarrowing were assessed as were eye posi-

tions, lip tone, tone of the masicatory muscles, and conscious proprioception (Greene and Oliver, 1993).

Auditory and visual evoked potentials

Ninety days following FIV-PPR inoculation, ketamine hydrochloride (Ketalar, 10 mg/kg IM) anesthetized cats maintained at normal body temperature with a heating pad were acutely aseptically fitted with subcutaneous monopolar needle electrodes (TECA Intropak DMG-50) to differentially record brainstem auditory evoked potentials (BAERs) and visual evoked potentials (VEPs). The two active electrodes were placed centrally near the vertex and on the midline over the frontal sinus, respectively. The ground electrode was placed in the nuchal muscles. For BAERs, cats were then fitted with bilateral polyethylene ear tubes placed into the external auditory canal. A 'Y'-connector attached the two tubes to the central sound source (Grass Instruments Audio Amplifier). A computer program, using National Instruments software ('LabView') and written for the MacIntosh II microcomputer, was used to generate the stimuli and to collect the data. The sound stimuli consisted of a 70 dB SPL condensation produced 'click' generated by a 100 ms input to the audio amplifier. Raw signals obtained within the first 10 ms post stimulation were amplified by a Grass P-511 instrumentation amplifier filtered between 300–3000 Hz and computer averaged. Stimuli were delivered bi-aurally at the rate of 10 Hz for a total of 1024 stimuli. On-line averaging of the signals allowed us to repeat trials within a recording session to determine stability of the evoked responses.

For visual evoked events (VEPs) stimuli were elicited by a Grass clinical photostimulator set on the highest intensity setting #12. Cats were biocularly stimulated at a rate of one stimulus flash/second with the light source 30 cm from the nose. Responses obtained within 200 ms post stimulation to 100 stimuli were averaged for each recording session. One replication was done for each experiment to evaluate stability of the evoked events. Ambient room lighting remained on. As with auditory stimulation, the visual stimuli and data acquisition were controlled by a MacIntosh II computer.

Evoked events were analysed off-line using signal averaging capabilities of a Mac II microcomputer using custom-made LabView software. Averaged peak latencies and amplitudes were calculated and compared individually between animals and as group means using the Student *t*-test (BAER, VEP) and nonparametric statistical tests (ESCP).

Spinal and peripheral nerve conduction velocities

Atropine sulfate (0.04 mg/kg body wt, SQ) was given as a premedication, and anesthesia was induced and maintained with inhaled halothane (1.5–2%). During evoked spinal cord potentials (ESCP) recording, muscle paralysis was achieved using atracurium besylate (0.2 mg/kg body wt, IV). The cats were intubated, and during periods of paralysis, maintained on a respirator. Body temperature was maintained above 33°C with the use of a water blanket heating mattress.

Percutaneous recordings and stimulations were achieved via needle electrodes. Stimulating electrodes (TECA model MG25) were placed in close proximity to the sciatic nerve between the greater trochanter of the femur and the ischiatic tuberosity. Rectangular pulses, 0.05 ms in duration, were delivered at a rate of 5–10 pulses/s by a stimulator (Grass Instruments model 48) via a stimulus isolation and a constant current unit (Grass Instruments model PSIU6). Recordings at L6–7 were visually monitored to ensure that a maximal evoked response was produced with each stimulus.

ESCPs were recorded at all intervertebral spaces between lumbar vertebra 7 and thoracic vertebra 11 (L6–7 cranially to T11–12), between the last cervical and the first thoracic vertebrae (C7–T1), and at the atlanto-occipital junction (A–O). An active recording needle electrode (TECA Model MG 25; MG37 at the C7–T1 recording site) with a 1 mm bared tip was placed percutaneously through the interarcuate ligament. A reference electrode (model MG25) with a 2–3 mm bared tip was placed in the epiaxial muscles 1–2 cm directly lateral to the active electrode. A ground electrode was placed subcutaneously within 5 cm of the recording site.

Each stimulus-evoked compound action potential was detected by a high impedance probe (Grass Instruments Co model HIP5) and fed into a differential amplifier (Grass Instruments Co model 511K), filtered with a band pass of 3 Hz–10 KHz. The amplified signal pulses were led into an oscilloscope and an analog-to-digital converter (Mac Adios II, GW Instruments, INC). The digitized signal was then fed into a computer where it was averaged and stored. Each averaged evoked potential was produced by averaging 256 to 1024 successive responses to sciatic nerve stimulation. Each evoked potential that was averaged contained 1024 data points, with a sampling period of either 7 or 10 uS/point.

Motor and sensory nerve conduction velocities (MNCVs and SNCVs) were also tested. An active 4 mm diameter silver-chloride silver disk electrode was placed in contact with electrical conducting gel on bare skin over the interosseous muscles on the plantar surface of the metatarsal region of the foot. A similar reference electrode was placed on bare skin between the third and fourth digits of the foot. The tibial nerve was stimulated distally just deep to the common calcaneal tendon, and proximally in the popliteal region. The ground needle electrode was placed under the skin between the stimulating and

recording electrodes. Evoked muscle potentials were recorded by the previously described electrodes. The medial branch of the superficial radial nerve was used to determine the SNCV. Percutaneous stimulating needle electrodes were placed distally along the nerve as it courses along the cephalic vein. A percutaneous active needle electrode with a 1 mm bared tip was placed next to the nerve as it emerges from under the lateral head of the triceps muscle, and the reference electrode with a 3 mm bared tip was placed under the skin approximately 1–2 cm caudal to the active electrode. The ground electrode was placed under the skin midway between the stimulating and recording electrodes. Duration of the sensory evoked potential was also examined. Because the evoked potential will increase in duration with an increase in nerve length, we divided the duration of the potential (in ms) by the length of the nerve (in meters). This results in a duration factor that compensates for differences in sizes of the cats and length of the nerves measured.

Distances used in calculating peripheral conduction velocities were obtained by using a flexible ruler and measuring the course of the nerve as it traverses under the skin. Distances for spinal cord conduction velocities were derived by taking measurements of the cervical (AO to CT), thoracic (CT to T13–L1), and lumbar (thoracolumbar junction to L3–L4) regions from a lateral radiograph.

These electrophysiologic techniques have been previously used in other adult, SPF cats. When comparing the results of one recording session with the results obtained from the same cat as much as one year later, there has been very little variation (generally $<10\%$) in conduction velocities (data not shown). Therefore, in this study, any variation greater than 20% from one recording session to the next has been deemed abnormal. Likewise, those conduction velocities greater than two standard deviations below the control mean were also considered abnormal.

Sleep studies

One cat, from each of the three treatment groups, was anesthetically induced by Ketalar, intubated, and maintained under halothane anesthesia (2–3% in oxygen) surgically prepared for sleep recording by implanting cortical stainless steel electrodes in the right frontal and right parietal cranial vault. In addition, bipolar stainless screw electrodes were placed in the eye orbit to record eye movements. Bipolar nichrome leads (250 mm in diameter) insulated except for the wire tips were placed in the nuchal musculature to monitor electromyographic activity. A grounding electrode was placed in the left frontal bone. All leads were cemented to the cranial vault by dental cement and brought together in a connector assembly (amphenol) for later monitoring of electrical activity. Cats were given 2 weeks of recovery and subsequently acclimatized a minimum of 3 days to an environmental chamber (BRS/LVE large animal cubicle-35′H × 34′W × 29′D) containing a 2 ft × 2 ft rug and a litter pan, prior to sleep recording. Two channels of electroencephalographic activity were recorded on a Grass model 78 polygraph in addition to one channel each of electromyographic and eye movement activity. EEG activity was simultaneously recorded on an instrumentation tape recorder (Vetter) for later off-line analysis of EEG frequency spectra.

EEG data analysis included the initial visual inspection of the recorded channels of the EEG to determine on-going quality and appearance of spontaneous waveforms. Further analysis included the determination of latency, duration, and percent of total sleep time of discrete sleep states. These measures were calculated and displayed off-line using computer software (Somnibus).

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