Sleep patterns are disturbed in cats infected with feline immunodeficiency virus

OSCAR PROSPERO-GARCIA*, NICOLE HEROLD*, TOM R. PHILLIPS*, JOHN H. ELDER†, FLOYD E. BLOOM*, AND STEVEN J. HENRIKSEN*†

Departments of *Neuropharmacology and †Molecular Biology, The Committee for Sleep Disorders Research, The Scripps Research Institute, La Jolla, CA 92037

Contributed by Floyd E. Bloom, September 19, 1994

ABSTRACT Human immunodeficiency virus (HIV)-related sleep disturbances have been reported early in AIDS. Likewise, the feline immunodeficiency virus (FIV), a natural lentivirus pathogen of cats, produces a similar immunodeficiency syndrome with neurological sequelae. To identify the neurophysiological substrate of FIV infection in brain, pathogen-free cats were infected with the Maryland strain of FIV. Eight weeks after inoculation, all FIV-infected cats seroconverted and virus was detected in the cerebrospinal fluid and in the mononuclear cells of peripheral blood. Ten to 12 months after the FIV inoculation, inoculated and control cats were surgically implanted with electrodes to record the sleep/wake cycle. These sleep recordings were obtained under conditions controlling for environmental variables and instrumental adaptation. FIV-infected cats spent 50% more time awake than the sham-inoculated controls and exhibited many more sleep/waking stage shifts—i.e., 40% more than controls. In addition, FIV-infected cats showed 30% of rapid eye movement (REM) sleep reduction compared to controls. The latency to sleep and REM sleep onset was also significantly delayed in FIV-infected cats. In addition, a remarkable increase in cortically recorded spindle activity (8–13 Hz) was observed during slow-wave sleep in some infected subjects, similar to changes described in HIV-infected humans. Moreover, infected cats exhibited no overt signs of systemic morbidity, such as hyperpyrexia or body weight loss. These results indicate that FIV-infected cats exhibit sleep abnormalities similar to the sleep disturbances previously described in AIDS patients and further support the feline preparation as a valuable animal model of HIV infection of the central nervous system.

The early entry of human immunodeficiency virus (HIV) into the central nervous system (CNS) (1) causes neuropsychiatric symptoms, which are often the first clinical manifestation of AIDS (2).

Systematic studies seeking to understand the pathogenesis of brain changes during HIV infection and AIDS have demonstrated that specific changes in sleep architecture may precede the first neuropsychiatric symptoms. In fact, Norman et al. (3) have described that HIV+ patients show distinctive changes of sleep patterns in that slow-wave sleep was specifically increased during the second half of the night. Moreover, they reported an increase in the number of overall awakenings throughout the night and an increase in the amount of electroencephalographic (EEG) frequencies in the 8- to 13-Hz range appearing during slow-wave sleep. In contrast, Kubicki et al. (4) have described a reduction in slow-wave sleep and rapid eye movement (REM) sleep in more severely compromised AIDS patients. A correlation between absolute CD4+ cell counts and an increase of sleep complaints has been described in asymptomatic HIV+ individuals (5, 6).

To examine the lentivirus-derived pathophysiological sequelae in the CNS, we have used an animal model of AIDS, the feline immunodeficiency virus (FIV)-infected cat. FIV, a closely related member of the family Lentiviridae to which HIV belongs (7–9), is a natural pathogen of domestic cats, producing a syndrome similar to that caused by HIV in humans (10). FIV mimics HIV cellular tropisms, invading the CNS a few weeks after experimental inoculation (11), and produces several neurological and electrophysiological disturbances (12, 13) similar to the alterations observed in humans (3, 14).

In this study, we analyzed the sleep/wake cycle of FIV+ cats that exhibited a reduction in CD4+ counts and also demonstrated several neurological and electrophysiological abnormalities (13).

MATERIALS AND METHODS

Subjects. Eight specific pathogen-free (SPF) cats were obtained from Liberty Laboratories (Liberty Corners, NY). Five SPF cats were intravenously inoculated with 1000 TCID50 units of the Maryland strain of FIV at 20 weeks of age (see ref. 13). This viral strain was originally obtained from a young (2-year-old) FIV-infected cat free of known other viral pathogens including feline leukemia virus (12). The FIV virus was isolated and amplified by cocultivating peripheral blood mononuclear cells from this cat with those from SPF cats. Control cats were sham-inoculated with tissue culture medium of uninfected cells. Samples of blood and cerebrospinal fluid (CSF) were taken serially after inoculation to determine the presence of the virus in these cats. FIV antibodies and CD4+ cell determinations were also performed as described (13).

Felies were group housed in two rooms, with FIV-infected cats separated from sham-inoculated subjects. Both groups were allowed ad libitum access to food and water. The dark/light cycle was controlled (12:12, lights on at 6:30 a.m.). Both groups were physically examined at frequent intervals for gross general and neurological signs. Core temperatures were evaluated daily with a rectal thermistor probe.

Surgical Procedures. At 10–12 months after the inoculation with FIV, control and infected cats were pretrained for anesthesia with ketamine hydrochloride (7 mg/kg) and atropine sulfate (0.04 mg/kg) and then were intubated and maintained under anesthesia with halothane (0.5%). A set of stainless steel screw electrodes was placed in the parietal and

Abbreviations: HIV, human immunodeficiency virus; CNS, central nervous system; REM, rapid eye movement; FIV, feline immunodeficiency virus; CSF, cerebrospinal fluid; EEG, electroencephalogram; EOG, electrooculogram; EMG, electromyogram

To whom reprint requests should be addressed: Department of Neuropharmacology CVN-13, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.
RESULTS

Virus Isolation, FIV Antibodies, and CD4 Lymphocyte Determinations. FIV was recovered from serum, CSF, and peripheral blood mononuclear cells of the infected cats. Anti-FIV antibodies were also detected in the serum of all FIV-infected subjects by week 8 of infection. All the controls remained seronegative. A decrease in circulating CD4+ lymphocytes was also seen in the FIV cats (13).

Physical Examination. Early in the postinoculation period, delayed righting and pupillary reflexes (by 8 weeks postinfection) and anisocoria (by 12 weeks) were detected. However, these signs were expressed intermittently throughout the next 15 months of observation. Miscellaneous skin infections, including cutaneous ulcers, acne, etc., were observed in some of the FIV cats (see ref. 13). No abnormal changes in the core temperature or body weight of either group were detected.

Behavioral Signs. During the physical examination and during the recording periods, a growing neophobia and reticence of handling were noticed in FIV-infected cats—i.e., lack of affection, fearfulness, and nonaggressive escape behavior. These signs were observed to a greater or lesser degree in all FIV-infected cats.

EEG Changes. Visual inspection of the EEG of the FIV-infected cats suggested an increase in the appearance of spindle activity, an EEG frequency between 8 and 13 Hz (Fig. 1), during slow/wave sleep (non-REM, delta sleep). Further quantification showed a remarkable increase (>100%) of spindle density in the infected cats compared to the sham-inoculated controls (mean ± SEM of number of spindles per min; normal, 1.73 ± 0.06; FIV cats, 3.62 ± 0.51; P = 0.03).

Sleep Architecture Disturbances. The sleep/waking cycle was classified on the bases of the EEG, EOG, and EMG in four states: waking, slow-wave sleep 1, slow-wave sleep 2, and REM sleep. This sleep/waking cycle classification is based on standardized criteria (15). Visual inspection of the hypnograms (Fig. 2) provides clear evidence of sleep disruption in the FIV-infected compared to the sham-infected cats. Comparison of the FIV group with the control group demonstrated an increase of the total time spent in wakefulness (50%). Likewise, FIV cats exhibited a substantial reduction in REM sleep (30%). In addition, the latency to sleep onset as well as latency to the first REM sleep period was delayed (Fig. 3). The reduction in total time spent in REM sleep was

![EEG of FIV cats during slow-wave sleep showed intrusion of fast (spindle, 10–13 Hz) activity. This activity is seen in normal cats with less frequency of appearance.](image-url)
Fig. 2. Illustration of the sleep architecture of three normal (left hypnograms) and three FIV-inoculated (right hypnograms) cats. Differences in the sleep architecture between the two groups of animals are evident. There is a prolonged latency to sleep onset and to the first REM sleep episode as well as reduction in REM sleep frequency. W, wakefulness; S1, slow-wave sleep 1; S2, slow-wave sleep 2.

a result of a combined decrease in the frequency and duration of the individual periods (Table 1).

**DISCUSSION**

We report here that discrete, consistent, and reproducible sequences of electrophysiological changes occur in the brains of FIV-infected cats within a few months after inoculation and well before secondary physical changes related to immunodeficiency have arisen. Thus, polysomnographically demonstrable augmentation of EEG spindle activity, increased waking, and reduced REM sleep were seen in every FIV-inoculated cat by month 12 after inoculation. These objective CNS changes followed other neurological signs in gait and pupillary regulation, as well as softer behavioral indications of alterations in interanimal interaction. The occurrence of these physical and neurological changes, along with the presence of nascent FIV and the detection of anti-FIV antibodies in blood and CSF as well as a substantial reduction in CD4+ lymphocytes, all document the diagnosis of FIV disease. These FIV-infected cats have also shown abnormal peripheral nerve, spinal cord, brainstem, and cerebrocortical electrical responses evoked by auditory, visual, and somatosensory stimulation (ref. 13; D. Wheeler, O.P.G., T.R.P., J.H.E., F.E.B., and S.J.H., unpublished data).

Both EEG and sleep disturbances have been described in HIV+ and AIDS patients as a primary sign of this disease (3, 4, 16). In our study, sleep recordings were made ~12 months after virus inoculation, a time when these cats exhibited no overt clinical signs of the AIDS-like disease. Our observations confirm findings in humans and support the notion that sleep abnormalities can be detected early in the progression of the disease (3). However, additional work is required to determine how early after FIV infection these sleep changes can be detected.

Although the underlying pathophysiology of these sleep disturbances is unknown, several investigators have described immunological changes that accompany the progression of the disease in humans that may account, in part, for the sleep alterations. For example, there is a substantial elevation of the cytokines interleukin 1, interleukin 6, and tumor necrosis factor α (TNF-α) in the CSF of HIV+ patients (17). These cytokines, when administered intraventricularly, have been shown to alter sleep patterns in several animal models (18). Moreover, a positive correlation between the cyclic variations in blood levels of TNF-α and EEG delta
(0.5–4.5 Hz) power spectrum during sleep has been described in humans. This correlation is disrupted in HIV+ subjects (D. F. Darko, C. Gallen, S. J. Brown, R. Hyduk, J. White, J. Koziol, J. Hampton Atkinson, D. T. Munnell, P. Naitoh, J. D. Assmus, J. Allen McCutchan, and M. M. Mitler, personal communication).

Alternatively, it has been suggested that another mechanism by which HIV may produce brain pathology is through the release of viral envelope glycoproteins—for example, the surface glycoprotein of the viral envelope (SU-Env), also known as glycoprotein 120 (19). SU-Env may exert its effect by activating glutamatergic receptors (19) or by the release of compounds with glutamate-agonist activity, like quinolinic acid. Indeed, an augmentation of quinolinic acid has been reported in the CSF of patients with AIDS dementia (20) and in monkeys infected with simian immunodeficiency virus (21). Furthermore, the infusion of a high concentration of glutamate into the dorsal mesopontine area increases waking and reduces REM sleep in cats (22). Brainstem cholinergic systems were implicated in this selective effect on REM sleep (22). Interestingly, another investigation has suggested that human SU-Env glycoprotein binds to cholinergic receptors (23).

We have purified SU-Env from FIV and administered it into the brain ventricles of rats (24). The glycoprotein (200 ng) produced an increase in wakefulness and a decrease in REM sleep during the postinjection recording period. Slow-wave sleep was also reduced during the first 2 hr. This finding supports the possibility that the disturbances observed in the FIV cats and in HIV+ humans may be caused by the direct or indirect actions of SU-Env.

In summary, FIV-infected cats exhibit immunological as well as electrophysiological abnormalities similar to what has been observed in HIV+ and AIDS patients. We suggest that utilization of the FIV model provides a unique opportunity to study the mechanisms by which this retrovirus damages the brain.

We wish to thank Dr. Robert Olmsted for providing FIV Mount airy for these studies. We also wish to thank Dr. Elizabeth Ford for surgical assistance and post surgery care of the cats and Miss Jodi Everitt for her assistance in the handling of the cats. This work was supported by Grant P50MH47680 from the National Institute of Mental Health.

Table 1. Total shifts of sleep states and REM sleep latency and duration (mean ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>Sham inoculated</th>
<th>FIV inoculated</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total shifts</td>
<td>106 ± 9.2</td>
<td>120.8 ± 10.8</td>
<td>+14.0</td>
</tr>
<tr>
<td>Waking shifts</td>
<td>29.7 ± 4.4</td>
<td>42.6 ± 4.8</td>
<td>+43.6</td>
</tr>
<tr>
<td>SWS1 shifts</td>
<td>34.3 ± 5.8</td>
<td>37.8 ± 6.6</td>
<td>+10.1</td>
</tr>
<tr>
<td>SWS2 shifts</td>
<td>31 ± 1.0</td>
<td>34.2 ± 6.0</td>
<td>+10.3</td>
</tr>
<tr>
<td>REM sleep shifts</td>
<td>11.0 ± 0.0</td>
<td>7.0 ± 1.9</td>
<td>−36.4</td>
</tr>
<tr>
<td>SWS-REM latency</td>
<td>49.9 ± 14.7</td>
<td>135.7 ± 43.5</td>
<td>+172.2</td>
</tr>
<tr>
<td>REM duration</td>
<td>7.4 ± 1.7</td>
<td>3.9 ± 1.0</td>
<td>−46.3</td>
</tr>
</tbody>
</table>

FIV-inoculated cats exhibited more sleep state shifts, suggesting less stability of sleep. This instability was a result of more awakenings. SWS-REM latency, latency from slow-wave sleep (SWS) onset to the first REM sleep onset.


