Influenza Viral Infections Enhance Sleep in Mice (43945)

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Abstract. **Sleepiness is a common perception during viral infection. Nevertheless, very little is known about the effects of viral infection on sleep. The aim of the present study was to test whether sleep was altered by influenza viral infection in mice. After 2–3 days of baseline sleep recordings, Swiss-Webster mice were infected intranasally with a lethal (H1N1) or a nonlethal (H3N2) strain of influenza virus. Sleep was recorded again for an additional 3 days. Non–rapid eye movement sleep (NREMS) was dramatically increased after inoculation of the H1N1 virus with a latency about 16 hr. Rapid eye movement sleep (REMS) was significantly suppressed after a longer latency. Both changes lasted until the end of the recording and occurred in both young (35-day-old) and adult (90- to 100-day-old) animals. Control animals did not show changes in sleep after sham infection with allantoic fluid. The H1N1 virus also caused dramatic decreases in body temperature and locomotor activities with a latency about 4–5 hr after viral inoculation. The H3N2 virus induced very similar changes in sleep, although the effects were much smaller in magnitude than those induced by the H1N1 virus, even though a much higher dose (10-fold) of the H3N2 virus was used. The present study shows that influenza viral infection induces profound and long-lasting increase of NREMS and suppression of REMS. These viral-induced changes in sleep likely represent a host-defense response.** [P.S.E.B.M. 1995, Vol 210]

Excessive sleepiness is a symptom found in nearly all infectious diseases; it is a central nervous system component of the acute phase response. For example, enhancement of non–rapid eye movement sleep (NREMS) and electroencephalographic (EEG) slow wave activity (SWA) occur during bacterial, fungal, or protozoan infections or after challenge by bacterial products (reviewed Ref. 1). Little is known, however, about sleep changes during the course of viral infection. Previously, we showed that NREMS and EEG SWA are enhanced for a few hours after intravenous (iv) injection of large doses of influenza virus into rabbits. However, influenza virus only undergoes a partial replication in rabbits (an abortive infection). Sleep responses occurring during a productive viral infection in mice have been reported in preliminary communications (2, 3). The objective of the present work was to characterize fully sleep changes during the course of influenza viral infection in mice and to test the hypothesis that the magnitude of the sleep responses is dependent upon the severity of infection as indicated by body weight loss, increase in lung weight, and general appearance. We report that both a lethal H1N1 and a nonlethal H3N2 strain of influenza virus induce long-lasting changes in sleep although responses to the H1N1 strain are greater.

Materials and Methods

Animals. For sleep studies, male viral antibody–free (VAF) Swiss-Webster juvenile mice were obtained from Taconic Laboratory (Germantown, NY) and adult VAF mice from Charles River Laboratories (Wilmington, CA). Mice were given a 1- to 2-week period to recover from shipping. They were maintained in an AAALAC accredited animal facility. Ta-

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ble I shows the number and age of mice, type of treatment, and type of data collected in each experiment. Experimental protocols were approved by the institutional animal care and use committee.

Virus Stocks. The A/PR/8/34-NS2 (H1N1) influenza virus used in the sleep studies was prepared by Dr. H. Maassab, University of Michigan (Ann Arbor, MI). This viral stock was grown in 10-day-old embryonated SPAFAS eggs and had a titer of 2×10^9 EID_{50}/ml. Control allantoic fluid was collected from uninoculated SPAFAS eggs, tested for bacterial contamination in blood agar plates and for pyrogenicity in rabbits; both assays gave negative results. The A/Port Chalmers/1/73 (H3N2) influenza virus used in the sleep studies was the gift of Dr. Parker A. Small, Jr., University of Florida, Gainesville, FL. Viral stocks were grown in 9- to 11-day-old embryonated SPAFAS eggs and had a titer of 1×10^8 EID_{50}/ml. Both viral strains were mouse-adapted as previously described (4), but the H1N1 strain caused lethality in mice beginning 4 days after intranasal inoculation with 10^4 EID_{50} or higher doses; the H3N2 strain failed to cause lethality at 10^7 EID_{50}.

Surgery. Mice were anesthetized subcutaneously with ketamine (25 mg/kg) and xylazine (25 mg/kg). Three stainless steel electrodes (Plastic One, Inc., Roanoke, VA) were implanted in the skull over the parietal cortex for electroencephalographic (EEG) recording. Three stainless steel electrodes (Plastic One) were implanted in dorsal neck muscles for electromyographic (EMG) recording. The electrodes with attached wires were fixed to the skull with dental cement. After surgery the animals were individually maintained in separate recording cages in a thermoneutral environmental chamber (Hotpack, Philadelphia, PA) with ambient temperature at 30° ± 1°C. A 12:12-hr light:dark cycle (light on at 5:00 AM and off at 5:00 PM) was maintained during the period of recovery from surgery and throughout the experiments. Food and water were freely available.

In Experiment 3, mice were anesthetized with ketamine (25 mg/kg) and xylazine (25 mg/kg). Each mouse was implanted with a VM-FM Mini-Miter weighing 0.5 g, (Mini-Miter Co., Inc., Sunriver, OR) in the abdominal cavity to monitor body temperature and locomotor activity by radiotelemetry from receivers placed under the mouse cages; each cage held one mouse.

Sleep Recording and Scoring. After surgery, animals were given a recovery period (3–6 days in Experiment 1, and 7 days in other experiments) and adapted to the recording system during the last 2 days of this period. In all sleep experiments sleep recording started at dark onset and lasted for 6 days. Between 10:00 and 11:00 AM on the third day recording was briefly discontinued and the mice were infected with virus or sham-infected with vehicle or diluted allantoic fluid. The first 2 days and 17 hr of recording served as baseline data, and the response to viral infection was recorded for 3 days and 6 hr.

The sleep data were collected with a Grass polygraph (Grass Instruments Company, Quincy, MA). The EEG signals were amplified with a 7P5 wide band EEG preamplifier and a 7P-DA-G DC driver. The one-half cutoff for low and high frequencies was set at 0.5 and 35.0 Hz, respectively. The EMG signals were amplified with a 7P511J amplifier with one-half cutoff for low and high frequencies set at 100 and 10,000 Hz, respectively. In the first experiment, the sleep data were recorded on paper. In the other experiments, data collection was controlled by a 386 microcomputer. The J6 output from the DC drivers or 7P511J amplifiers was fed into a 12-bit PC30D analog-to-digit (AD) converter (Omega Engineering, Inc., Stamford, CT). The AD converter digitized the EEG and EMG signals at 128 Hz. The digitized data were transferred to the computer and displayed on the computer monitor. An on-line fast Fourier transformation (FFT) was performed on EEG data for every 2 sec. The FFT analyses generated the power density values from 0.0 to 63.5 Hz at a 0.5-Hz resolution. The results of FFT were averaged for every 10 sec; they were subsequently displayed on the computer screen and used as an aid in the determination of vigilance states. To decrease the demand for storage space the original EEG and EMG data were not saved. Instead, the envelopes of EEG and EMG wave forms and the averaged power density values from 0.5 to 20.0 Hz were saved to the hard disk drive in 10-sec segments.

After data collection, sleep data were scored visually according to the following criteria: wakefulness (W) was identified by low voltage fast EEG and high

Table I. Animal and Viral Characteristics in Sleep and Motor Activity Experiments

Experiment	Age (days)	*n*	Treatment	Dependent variables
1	25–30	6	H1N1 (NS2) virus	Sleep
		5	Allantoic fluid	Sleep
2	90–95	8	H1N1 (NS2) virus	Sleep
3	75–85	6	H1N1 (NS2) virus	Body temperature and motor activities
4	90–95	7	H3N2 virus	Sleep

amplitude EMG, rapid eye movement sleep (REMS) by low voltage EEG with clear (6–10 Hz theta activities) and dramatic suppression of EMG with occasional muscle twitches, and non-REMS (NREMS) by high voltage and low frequency EEG and low amplitude EMG. Sleep was scored in 12-sec epochs in paper recordings and in 10-sec epochs in computer recordings. The behavioral state for each epoch was determined by the predominant state during the epoch. The data were further averaged each hour for each behavioral state for further statistical analyses. The results were expressed as percentage of time spent in each state.

Body Temperature and Motor Activity (Experiment 3). Body temperature and motor activity data were collected with the Mini-Miter recording system using DataQuest III software (Mini-Miter Company, Inc., Sunriver, OR). The data were transferred from the receiver via a MP-12 multiplexer and cable to a 8086 microcomputer and saved to the computer disk at a rate of 1 sample per 10 min for both temperature and motor activity data.

In Experiment 3, there was a 7-day recovery period after surgical implantation of the transmitter. Data collection started at 1700 hr at dark onset (12:12-hr light:dark cycle) and lasted for 6 days except during the period of viral inoculation between 10:30–11:00 (2 days and 17 hr after the starting time for data collection). Throughout the recovery period, the experiment mice were maintained in a thermoneutral environment (30° ± 1°C) with food and water freely available. After data collection, the temperature and motor activity data were averaged hourly. The motor activity data were normalized by logarithmic transformation to reduce variance.

Viral Inoculation for Sleep Studies. On day 3, after 2 days and 17 hr of baseline recording of sleep (Experiment 1, 2, and 4) or body temperature and motor activity (Experiment 3), mice were lightly anaesthetized (about 3 min) by inhalation of methoxyfluorane (Metofane, Pitman-Moore, Inc., Mundelein, IL) and inoculated intranasally with influenza virus or allantoic fluid between 10:30 and 11:00. In Experiment 1–3, the mice were infected with mouse-adapted H1N1 influenza virus (A/PR/8/34-NS2). Each infected mouse received 10^6 EID_{50} of virus in 50 μl of a 10^{-2} dilution of allantoic fluid in Hanks' balanced salt solution delivered with a 100-μl sterile pipette. The control animals received the same amount of diluted allantoic fluid. In Experiment 4, each mouse received 10^7 EID_{50} of nonlethal H3N2 influenza virus (A/Port Chalmers/1/73-H3N2) in 50 μl of undiluted allantoic fluid. After inoculation with virus or allantoic fluid, sleep (Experiment 1, 2, and 4) or body temperature and motor activity (Experiment 3) data were collected for an additional 3 days and 6 hr (Day 4, 5, and 6). After viral inoculation on Day 3 only 6 hr (11:00–17:00) remained; data from this period are provided in the figures but not included in the tables.

After the experiments, the mice were sacrificed by cervical dislocation or lethal dose of ketamine (75 mg/kg) and xylazine (75 mg/kg). Lungs were removed and visually inspected to verify the infection. In Experiment 2 and 4, the lung weights were also measured at the end of the experiments. Body weights were measured in Experiment 2 and 4 at the time of viral inoculation and at the end of the experiments.

Statistics. The sleep, body temperature, and motor activity data were analyzed with two-way analysis of variance (ANOVA) for repeated measures. The first independent variable was the treatment (baseline versus Day 4, 5, and 6 after inoculation), and the second was the time (daytime versus nighttime). The data from the first 6 hr after viral inoculation were analyzed with separate repeated ANOVA (compared with baseline data during the corresponding time period). If a significant effect was found, the Newman-Keuls test was used for post hoc analysis. The difference was considered significant if P was less than 0.05.

Results

Effects of H1N1 Virus on Sleep, Body Temperature, and Motor Activity. H1N1 viral infection induced very large changes in sleep in juvenile mice (about 4 weeks of age) (Fig. 1 and Table II). NREMS was significantly increased with a latency of about 16 hr after viral inoculation (treatment effect: $F[3,12] = 32.2$, $P < 0.001$; time effect: $F[1,4] = 38.863$). The increase of NREMS was more pronounced during the nighttime than daytime (treatment and time interaction: $F[3,12] = 14.466$, $P < 0.001$). Further post hoc analyses indicated that except during the 6 hr of day 3 and the night of day 4 (starting from Hour 7 after inoculation) NREMS remained significantly higher than baseline level until the end of recording. REMS was significantly suppressed (treatment effect: $F[3,12] = 5.691$, $P < 0.012$; time effect: $F[1,4] = 0.234$, not significant; interaction: $F[3,12] = 4.053$, $P < 0.05$). The suppression of REMS started about 20 hr after viral inoculation; however, it only became significant during the daytime of Day 5 ($q[7,12] = 5.324$, $P < 0.05$) and Day 6 ($q[8,12] = 5.39$, $P < 0.05$). As a result of the dramatic increase of NREMS during the night and the decrease of REMS during the day, the normal circadian rhythm of sleep and wakefulness disappeared during the late stage of infection. In contrast, the control mice did not show any change in sleep after sham-infection with allantoic fluid (Fig. 2 and Table II).

Adult mice (about 13 weeks in age) displayed very similar changes in sleep after H1N1 viral infection

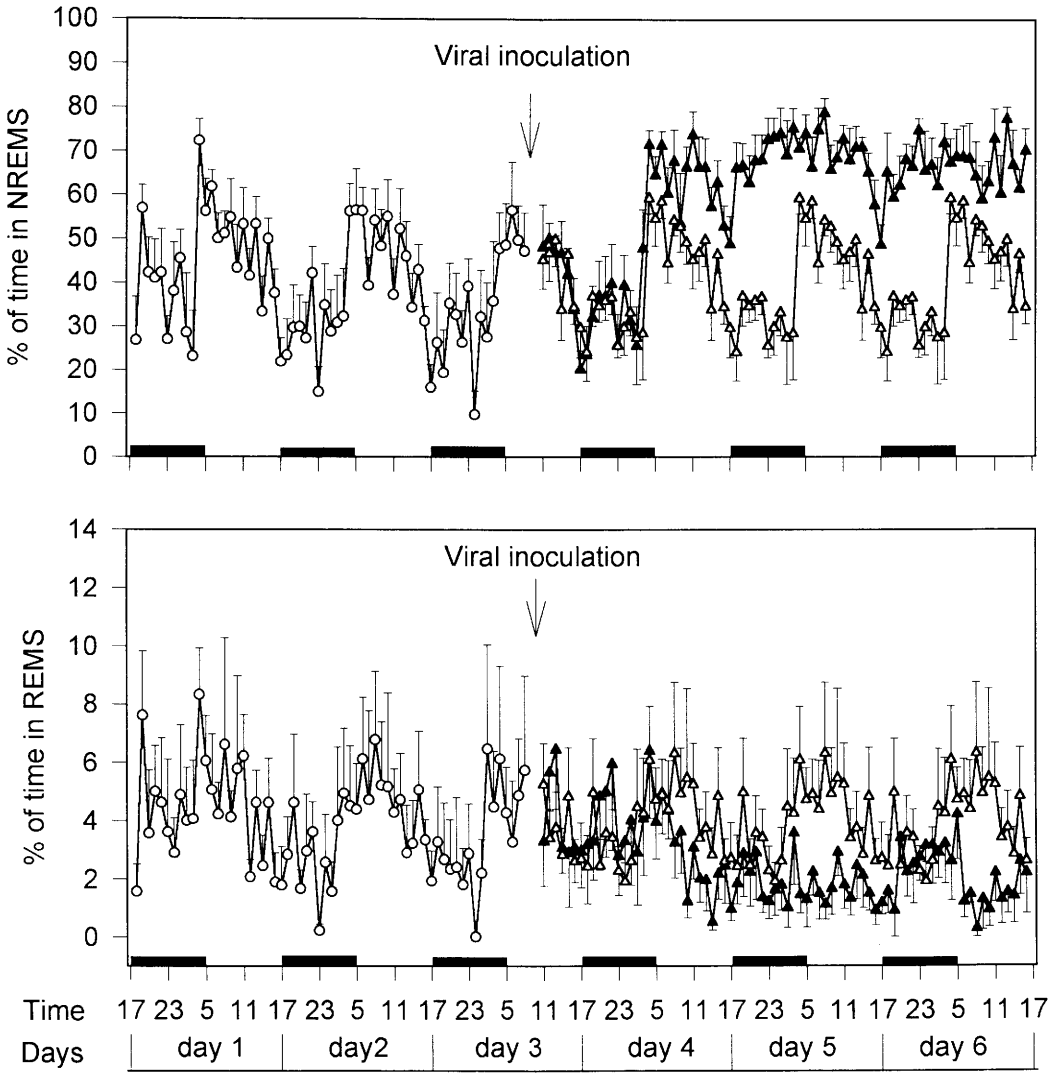

Figure 1. Effects of H1N1 viral infection on non–rapid eye movement sleep (NREMS) (top) and rapid eye movement sleep (REMS) (bottom) in young (30–35 days old at the beginning of the experiment) mice. Open circles represent baseline data. Filled triangles represent data collected after viral inoculation. Open triangles represent the repeated plot of averaged baseline data collected during the first 3 days of recording. The black bars indicate the dark period.

(Fig. 3 and Table III). The increase of NREMS started with a latency of about 15 hr after viral inoculation and lasted until the end of the experiment. However, the magnitude of this increase became smaller by the last day of recording. Repeated ANOVA confirmed that there was a significant treatment effect ($F[3,18] = 10.233$, $P < 0.001$), time effect ($F[1,6] = 20.231$, $P < 0.004$), and interaction ($F[3,18] = 19.901$, $P < 0.001$). The Newman-Keuls test further indicated that except during the 1st day postinoculation (consisting only of 6 hr) and the night of Day 5, NREMS was significantly increased compared with baseline level (daytime of Day 4: $q[6,18] = 6.718$, $P < 0.01$; Day 5: $q[4,18] = 7.482$, $P < 0.01$; Day 6: $q[3,18] = 5.750$, $P < 0.01$). REMS displayed just the opposite change after viral inoculation. Compared with baseline, REMS was decreased during the daytime of Day 4 ($q[6,18] = 9.522$, $P < 0.01$), entire Day 5 ($q[4,18] = 8.577$, $P < 0.01$), and Day 6 ($q[3,18] = 8.531$, $P < 0.01$), but not during Day 3 or the night of Day 4. One infected mouse died on the last day of the experiment in each of the first two experiments. These mice displayed essentially the same sleep responses to H1N1 viral infection as those that survived until the end of the experiments. However, the sleep data from these mice were excluded from statistical analyses because of the nature of repeated ANOVA.

Viral infection significantly decreased body temperature (treatment effect: $F[3,15] = 20.766$, $P < 0.001$; time effect: $F[1,5] = 5.657$, ns; interaction: $F[3,15] = 5.145$, $P < 0.02$) and motor activity (treatment effect: $F[3,15] = 21.447$, $P < 0.001$; time effect: $F[1,5] = 4.442$, ns; interaction: $F[3,15] = 3.995$, $P < 0.03$). Both decreases started about 4–5 hr after viral inoculation and lasted until the end of the experiment (Fig. 4 and Table IV). The decrease of body tempera-

Table II. Effects of H1N1 Virus or Sham Infection on Sleep in Juvenile Mice (Experiment 1)

	Baseline	Day 4	Day 5	Day 6
Virus (n = 6)				
NREMS				
24 hr	41.0 ± 1.6	49.9 ± 1.8[a]	67.3 ± 3.9[b]	65.9 ± 4.2[b]
Night	35.3 ± 2.0	37.1 ± 2.6	66.5 ± 3.8[b]	65.0 ± 4.4[b]
Day	46.6 ± 2.7	62.8 ± 2.4[b]	68.2 ± 4.3[b]	66.7 ± 4.6[b]
REMS				
24 hr	4.3 ± 1.4	3.8 ± 1.2	2.0 ± 0.7[a]	2.1 ± 0.6[a]
Night	3.8 ± 1.2	4.6 ± 1.5	2.2 ± 0.9	2.5 ± 0.7
Day	4.9 ± 1.5	3.1 ± 0.8	1.8 ± 0.6[a]	1.7 ± 0.5[a]
Sham (n = 5)				
NREMS				
24 hr	39.1 ± 1.80	38.3 ± 2.3	38.2 ± 2.5	36.0 ± 2.6
Night	30.7 ± 2.6	30.0 ± 3.1	29.0 ± 2.6	27.7 ± 3.9
Day	47.4 ± 2.7	46.6 ± 2.4	47.2 ± 2.6	44.2 ± 2.6
REMS				
24 hr	4.6 ± 1.0	4.3 ± 0.9	3.9 ± 0.8	3.9 ± 0.7
Night	3.3 ± 0.9	3.8 ± 1.2	3.1 ± 0.8	3.1 ± 1.0
Day	5.8 ± 1.1	4.9 ± 0.9	4.7 ± 0.9	4.7 ± 0.8

Note. Amount of sleep (mean ± SEM) is expressed as percentage of time spent in NREMS and REMS.
[a] $P < 0.05$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.
[b] $P < 0.01$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.

ture was dramatic, dropping from 37.03°C (average of baseline) to 35.11°C on Day 4 ($q[3,15] = 9.355$, $P < 0.01$), 35.56°C on Day 5 ($q[2,15] = 7.177$, $P < 0.01$), and 35.00°C on Day 6 ($q[4,15] = 9.888$, $P < 0.01$).

Effects of H3N2 Virus on Sleep. NREMS also significantly increased during H3N2 viral infection ($F[6,18] = 10.977$, $P < 0.001$) (Fig. 5 and Table V). This increase started 6–8 hr after viral inoculation and remained above the baseline thereafter (+14% during Hour 3–6 after infection, $P < 0.05$; +23.18% during Day 4 [starting from Hour 7 after infection], $q[3,18] = 6.759$, $P < 0.01$; +20.91% during Day 5, $q[2,18] = 6.097$, $P < 0.01$; and +23.70% during Day 6, $q[4,18] = 6.910$, $P < 0.01$). Two-way ANOVA indicated that the effects of H3N2 viral infection on REMS depended on time (treatment effect: $F[3,18] = 0.096$, ns; time effect: $F[1,6] = 4.178$, ns; interaction: $F[3,18] = 10.857$, $P < 0.001$]. The Newman-Keuls test showed that REMS was significantly decreased only during the light period on Day 4 (−38.57%, $P < 0.05$) and returned to the baseline level thereafter.

Swiss-Webster mice infected with A/PR/8/34-NS2 H1N1 virus showed external signs of disease (e.g., ruffled fur, and hunched posture). H1N1 virus-infected mice showed conspicuous loss of body weight (e.g., the body weight of the mice in Experiment 2 decreased by 21% at the end of experiments, $t[6] = 14.45$, $P < 0.01$) thereby confirming a previous report (5). Mice infected with H3N2 virus also showed a decrease in body weight (−14.0%, $t[6] = 4.78$, $P < 0.003$). The mice receiving H1N1 virus showed a significantly greater weight loss than those receiving H3N2 virus ($P < 0.05$). Necropsy on Day 7 showed that the lungs of all infected mice were hemorrhagic in patches or throughout. Lung weights (an index of lung edema) of the H1N1-infected mice were significantly greater than those from the H3N2-infected mice at the time of sacrifice (55 ± 0.03 vs 39 ± 0.02 mg, respectively).

Discussion

Influenza viral infection dramatically enhanced NREMS and suppressed REMS in mice for several days. The long-lasting increase in NREMS following viral inoculation found in the present study is consistent with the findings of Toth (3) and parallels the kinetics of viral shedding in the lungs of infected mice (6). In contrast, previous studies in our laboratory using iv injection of high doses of H1N1 into rabbits showed an increase of NREMS starting 1 hr after viral administration and lasting only for a few hours (7). Influenza virus undergoes only partial replication (abortive infection) in rabbits, while it undergoes a complete and sequential replication cycles (productive infection) in mice (8). It is possible that the time course and magnitude of sleep changes induced by the virus are dependent on the amount of viable virus in the infected animals. This interpretation is consistent with the observation that heat-killed virus does not induce any change in sleep in rabbits (7). In a preliminary study intranasal inoculation of heat-killed virus (A/PR/8/34-H1N1-NS2) also had no effect on sleep parameters in mice (n = 3). The long latency of sleep responses after H1N1 viral inoculation in mice corresponds to about two viral replication cycles, while the shorter latency of sleep responses after H3N2 viral inoculation corresponds to about one replication cycle (9). It is likely that the shorter latency of sleep re-

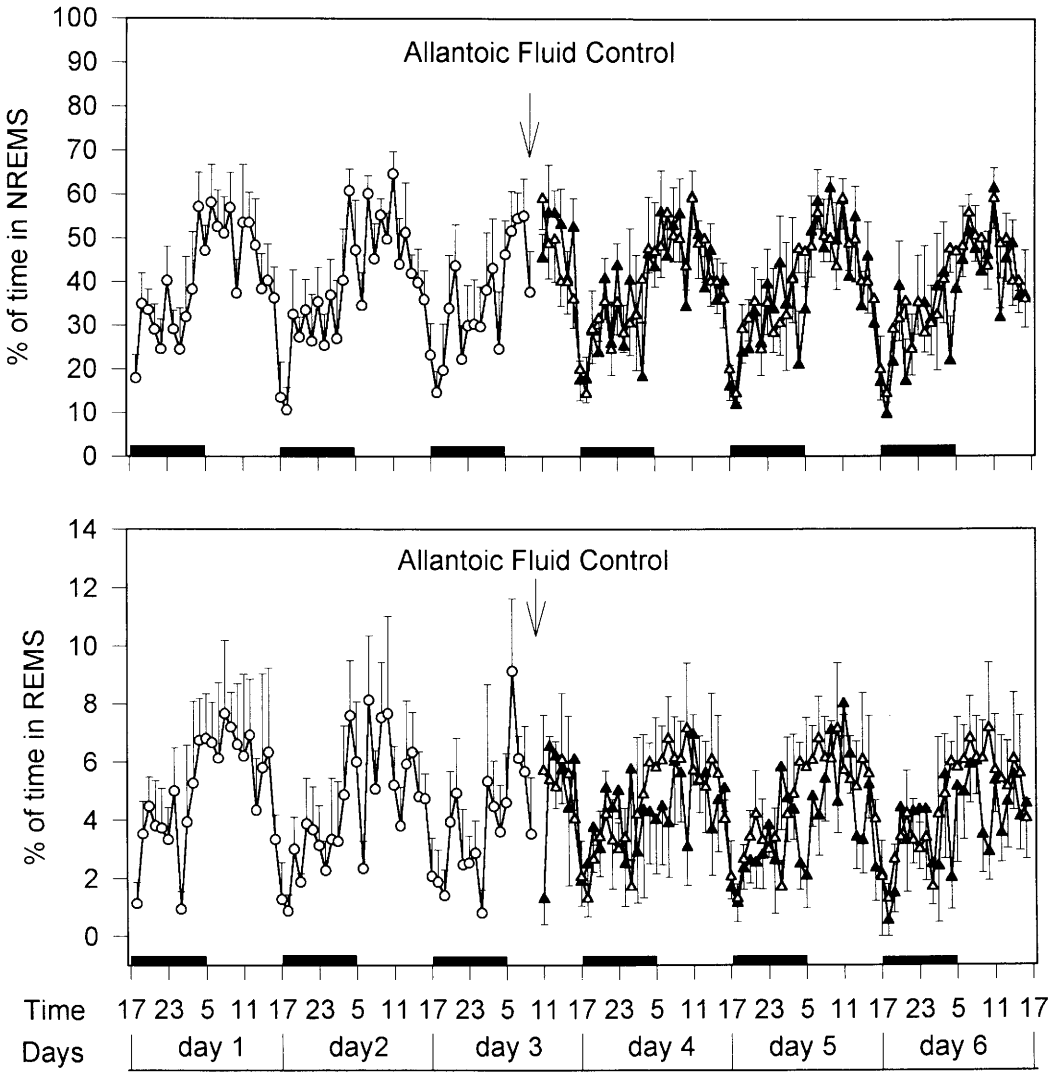

Figure 2. Effects of sham infection (allantoic fluid) on NREMS (top) and REMS (bottom) in young (30–35 days old at the beginning of the experiment) mice. Open circles represent baseline data. Filled triangles represent data collected after allantoic fluid treatment. Open triangles represent the repeated plot of averaged baseline data collected during the first 3 days of recording. The black bars indicate the dark period.

sponses after H3N2 infection resulted from the much higher dose of H3N2 virus used in the experiment.

Viral infections have been implicated in several sleep pathologies, including chronic fatigue syndrome (10, 11), mononucleosis (12), postviral fatigue syndrome (13), and sudden infant death syndrome (14). In humans, HIV (15) and influenza (16, 17) viral infections are also associated with changes in sleep. However, sleep was not measured throughout the course of the disease in any of these reports, nor was sleep of subjects characterized during periods of good health. The results reported here, coupled with the preliminary results previously reported (2, 3), are the first to systematically characterize sleep patterns over the course of any viral disease.

The mechanisms by which various viruses induce changes in sleep are not completely understood. It is clear on structural grounds that viruses must stimulate the host to induce changes in sleep by radically different mechanisms than bacteria, which induce sleep changes through their cell wall components (1). Carter and De Clercq proposed a general mechanism for viral toxicity with viral double-stranded RNA (dsRNA) as a key component (18). Such a mechanism can also be extended to explain the sleep changes induced by virus (reviewed in Ref. 1). The involvement of dsRNA in virus-induced sleep is indicated by a number of findings. Both the synthetic dsRNA poly(I:C) and isolated nuclease-resistant viral dsRNA from influenza virus-infected mouse lungs induce fever and altered sleep in rabbits (19). In addition, poly(I:C) induces a flu-like syndrome in humans (20). The involvement of dsRNA in responses to virus is further supported by the observation that pretreatment of rabbits with poly(I:C) blocks the acute phase response to influenza virus as does pretreatment with virus itself, thereby suggesting

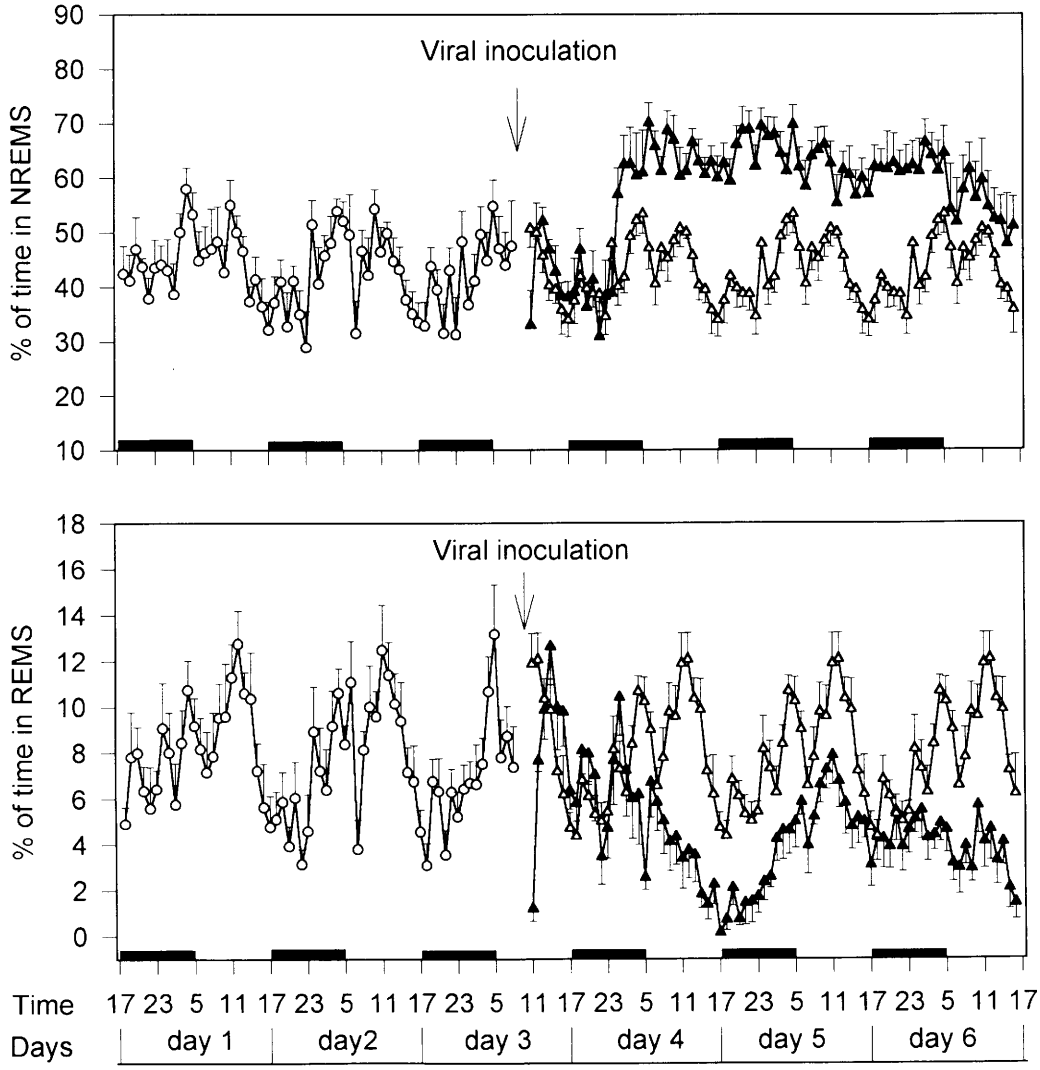

Figure 3. Effects of H1N1 viral infection on NREMS (top) and REMS (bottom) in adult mice. Open circles represent baseline data. Filled triangles represent data collected after viral inoculation. Open triangles represent the repeated plot of averaged baseline data collected during the first 3 days of recording. The black bars indicate the dark period.

Table III. Effects of H1N1 Viral Infection on Sleep in Adult Mice (n = 7) (Experiment 2)

	Baseline	Day 4	Day 5	Day 6
NREMS				
24 hr	43.0 ± 1.8	55.1 ± 1.4[a]	62.9 ± 2.7[a]	58.3 ± 4.8[a]
Night	41.3 ± 2.0	45.8 ± 1.5	65.1 ± 2.9[a]	61.3 ± 4.0[a]
Day	44.7 ± 1.9	64.5 ± 2.5[a]	60.8 ± 3.1[a]	55.3 ± 5.6[b]
REMS				
24 hr	8.0 ± 0.4	5.4 ± 0.7[a]	4.1 ± 0.5[a]	4.1 ± 0.6[a]
Night	6.8 ± 0.5	7.0 ± 1.0	2.2 ± 0.5[a]	4.7 ± 0.6[b]
Day	9.3 ± 0.6	3.9 ± 0.6[a]	5.9 ± 0.5[a]	3.6 ± 0.7[a]

Note. Amount of sleep (mean ± SEM) is expressed as percentage of time spent in NREMS and REMS.
[a] $P < 0.01$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.
[b] $P < 0.05$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.

that dsRNA can substitute for virus as a trigger of this cascade (21).

It is now clear that dsRNA, like bacterial cell wall products, induces production of many cytokines (22, 23), including interleukin-1α/β (IL-1α/β), interleukin-6 (IL-6), tumor necrosis factor-α/β (TNF-α/β), and α/β/γ-interferon (IFN-α/β/γ). Some of these cytokines have been detected in the lung washes of influenza virus infected mice (8, 24). These cytokines induce acute phase responses during viral infection, such as fever, suppression of food intake, social withdrawal, and reduced motor activity. IL-1α, IL-1β, TNF-α, TNF-β, and IFN-α are also somnogenic (1); it is thus likely that cytokines are common mediators for both viral- and bacterial-induced changes in sleep. This suggestion is consistent with the differential responses of sleep between high and low cytokine producing strains of mice: C57BL/6 (3) and Swiss-Webster mice (2), but

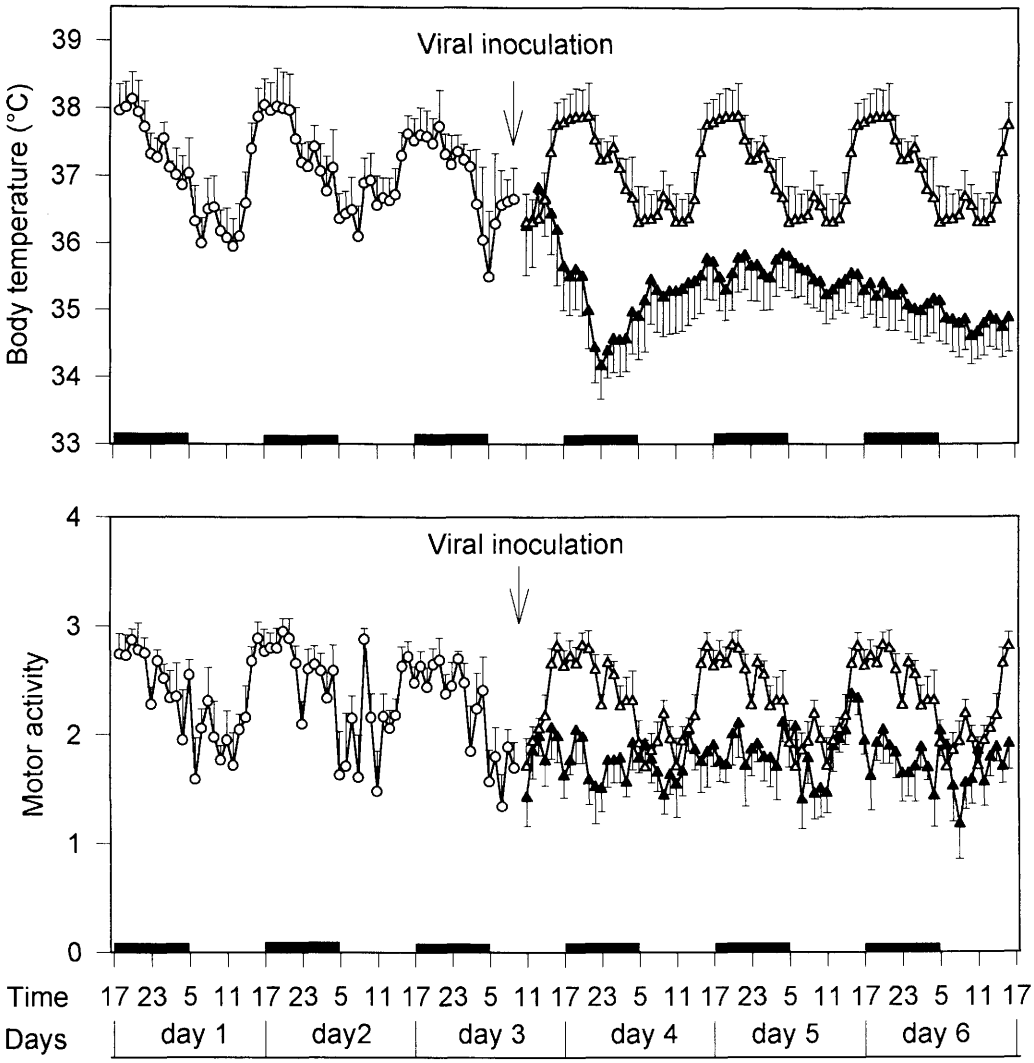

Figure 4. Effects of H1N1 viral infection on body temperature (top) and motor activities (bottom) in adult mice. Open circles represent baseline data. Filled triangles data collected after viral inoculation. Open triangles represent the repeated plot of averaged baseline data. The black bars indicate the dark period. The motor activity data are presented after logarithmic transformation of activity counts per hour.

Table IV. Effects of H1N1 Viral Infection on Body Temperature and Motor Activity in Adult Mice (n = 6) (Experiment 3)

	Baseline	Day 4	Day 5	Day 6
Body Temperature (°C)				
24 hr	37.0 ± 0.4	35.1 ± 0.5[a]	35.6 ± 0.5[a]	35.0 ± 0.4[a]
Night	37.4 ± 0.4	34.9 ± 0.5[a]	35.6 ± 0.5[a]	35.2 ± 0.5[a]
Day	36.6 ± 0.4	35.3 ± 0.6[b]	35.5 ± 0.4[a]	34.8 ± 0.4[a]
Motor Activity[c]				
24 hr	2.3 ± 0.1	1.7 ± 0.1[a]	1.9 ± 0.1[a]	1.7 ± 0.1[a]
Night	2.6 ± 0.1	1.7 ± 0.2[a]	1.9 ± 0.2[a]	1.8 ± 0.1[a]
Day	2.1 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.7 ± 0.1

[a] $p < 0.01$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.
[b] $p < 0.05$ compared with baseline, two-way repeated ANOVA and Newman-Keuls test.
[c] Motor activity is expressed as the logarithmic transformation of activity counts per hour.

not BALB/c mice (3), exhibit robust increases of NREMS following influenza viral inoculation.

In addition to dsRNA, neuraminidase, an influenza viral protein, is reported to induce IL-1 both *in vivo* and *in vitro* (25), and TNF-α production by macrophages *in vitro* (26). However, the enhanced cytokine production reported in these experiments may not be due to neuraminidase, since it is very labile and it was not determined whether the neuraminidase preparation used was contaminated with other microbial

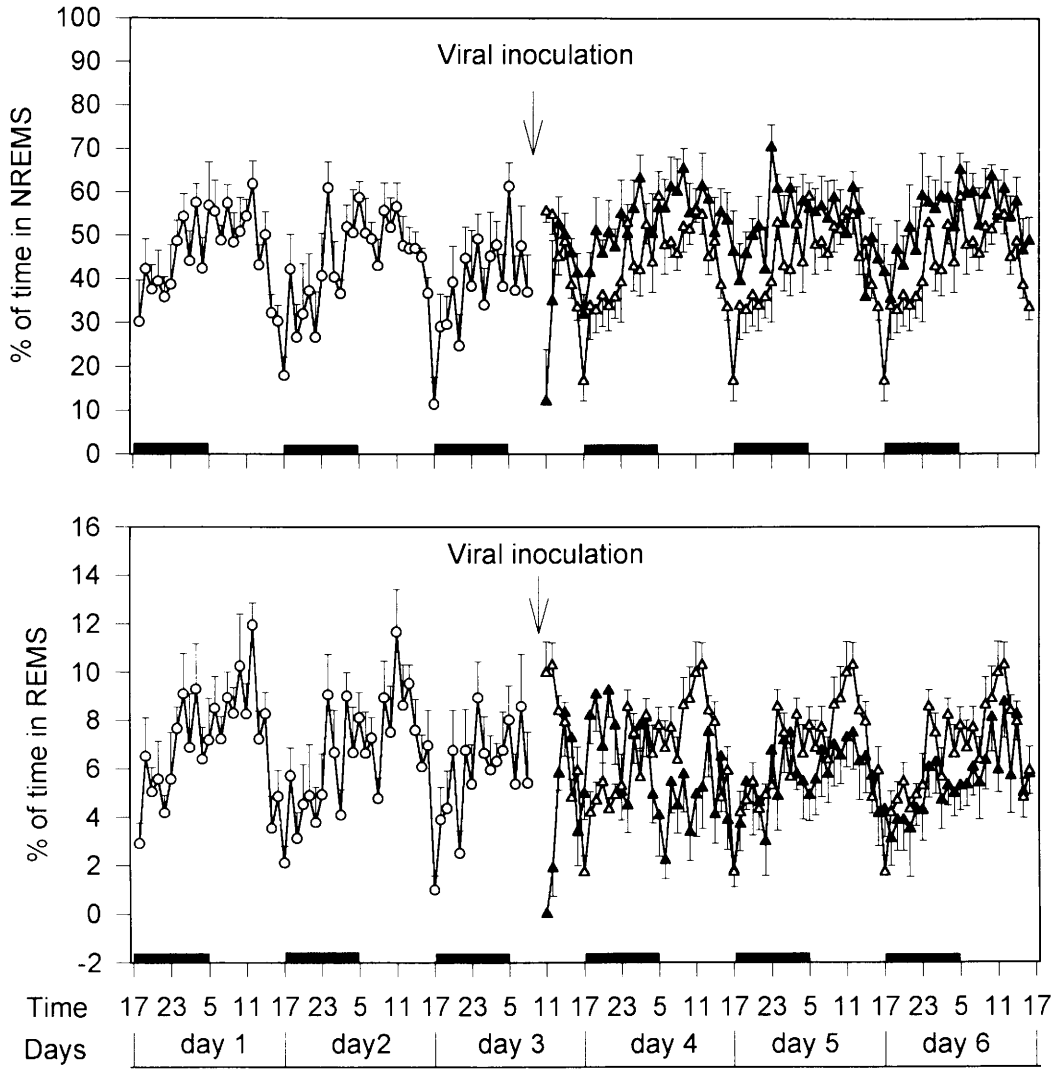

Figure 5. Effects of H3N2 viral infection on NREMS (top) and REMS (bottom) in adult mice. Open circles represent baseline data. Filled triangles represent data collected after viral inoculation. Open triangles represent the repeated plot of averaged baseline data collected during the first 3 days of recording. The black bars indicate the dark period.

products (reviewed Ref. 1). Further, purified enzymatically active neuraminidase (N9) in our hands failed to affect rabbit sleep or temperature (Kimura, Toth, and Krueger, unpublished data).

Sleep and body temperature regulation are closely related. For example, in NREMS there is a regulated decrease in brain temperature (27). Mild increases in either ambient or body temperature enhance sleep (reviewed Ref. 28). It is thus reasonable to ask if the sleep responses induced by viruses are secondary to the decreases in body temperature induced by the virus (29). This seems unlikely for several reasons. First, some substances (e.g., α melanocyte-stimulating hormone) induce hypothermia yet inhibit sleep (30) while others (e.g., prostaglandin E_2) induce hyperthermia and also inhibit sleep (31). After bacterial infection, hyperthermia occurs simultaneously with sleep responses (32). Fevers induced by cytokines (33) or bacterial products (34) can be blocked without affecting the sleep responses induced by these substances. In contrast, sleep responses to IL-1, but not IL-1-induced fevers, are blocked by inhibitors of nitric oxide production (35). In this study the temperature responses to H1N1 virus preceded the sleep responses by about 6–8 hr. Collectively, such data provide strong evidence that sleep and fever responses occurring during infection involve, in part, separate mechanisms and are not causative of each other.

It remains to be demonstrated directly experimentally whether the sleep responses to viral infection are beneficial to the host. There is, nonetheless, indirect evidence in support of this hypothesis. Brown showed that if mice immunized to influenza virus were sleep-deprived and rechallenged with influenza virus, they failed to clear the virus from their lungs (36). In another study, rats deprived of sleep for prolonged periods developed opportunistic bacterial infections (37). In studies involving inoculation of rabbits with either bacterial or fungal organisms, there was a correlation between sleep responses and clinical prognosis. Those

Table V. Effects of H3N2 Viral Infection on Sleep in Adult Mice (n = 7) (Experiment 4)

	Baseline	Day 4	Day 5	Day 6
NREMS				
24 hr	43.4 ± 3.2	53.5 ± 4.4[a]	52.5 ± 4.3[a]	53.7 ± 4.4[a]
Night	38.5 ± 4.2	49.6 ± 4.5[a]	52.1 ± 5.0[a]	50.6 ± 4.8[a]
Day	48.3 ± 2.4	57.4 ± 4.9[b]	52.5 ± 3.7	56.9 ± 4.2[b]
REMS				
24 hr	6.7 ± 0.4	5.6 ± 0.6	5.6 ± 0.2	5.4 ± 0.3
Night	5.6 ± 0.4	7.0 ± 0.5	5.1 ± 0.2	4.5 ± 0.3
Day	7.8 ± 0.6	4.8 ± 0.8[b]	6.1 ± 0.6	6.3 ± 0.5

Note. Amount of sleep (mean ± SEM) is expressed as percentage of time spent in NREMS and REMS.
[a] $P < 0.01$, two-way repeated ANOVA and Newman-Keuls test.
[b] $P < 0.05$, two-way repeated ANOVA and Newman-Keuls test.

animals exhibiting robust increases in NREMS early after challenge had a higher probability of surviving the infection (38). There are also several studies showing that either sleep deprivation affects one or more facets of the immune response or that changes in immune parameters are dependent upon sleep stages (reviewed in Ref. 1). For example, sleep deprivation, but not other stressors, primes for systemic production of streptococcal-induced tumor necrosis factor (39). Similarly, in another study the ability of lipopolysaccharide-stimulated monocytes to produce tumor necrosis factor and interleukin-1 was dependent upon the length of prior wakefulness (40). Regardless of whether sleep is linked to host defenses, current data clearly demonstrate that NREMS is dramatically enhanced over the course of viral disease.

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