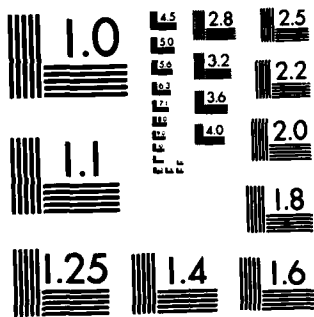AD-A170 804   PHARMACOLOGICAL RESETTING OF THE CIRCADIAN SLEEP-WAKE   1/1
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This research program developed strategies to reset the timing of the circadian (approximately 24-hour) sleep-wake cycle so that individuals could be maintained fully awake in any predetermined time in the 24-hour day. This capability would be of direct benefit in minimizing the deleterious effects of jet-lag and could promote alertness in facilities which must be staffed 24 hours a day.

The studies utilized a diurnal primate primate, the squirrel monkey (*Saimiri sciureus*), with a well defined circadian neurophysiology and sleep-wake physiology. This animal has a consolidated sleep-wake cycle that is comparable to that in humans.

During the three-year period of funding, the circadian sleep-wake organization in squirrel monkeys was characterized, and the rate of resynchronization after phase shifts of environmental light-dark cycles was determined. A number of different agents were (CONTINUED, OVER)

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ABSTRACT (Continued)

used to examine the mechanisms of sleep-wake cycle control including anisomycin, muramyl dipeptide, and sodium valproate. Anisomycin proved to have no net phase shifting effects when compared to saline controls; sodium valproate caused alterations in the circadian pacemaker period, and the third compound, muramyl dipeptide, induced sleep without altering circadian phase. Additional studies were undertaken using sleep deprivation techniques to examine the relative circadian regulation of the sleep-wake cycle in the squirrel monkey, and neurophysiological studies were undertaken to examine the effect of benzodiazepine and sodium valproate administration on the multiple unit activity of neural sites which display circadian rhythmicity. Further studies examined the circadian rhythms in benzodiazepine receptor activity and demonstrated that the rhythms were eliminated in SCN-lesioned animals.

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DEPARTMENT OF THE U.S. AIR FORCE

AIR FORCE OFFICE OF SCIENTIFIC RESEARCH

GRANT AFOSR 83-0194

PHARMACOLOGICAL RESETTING OF THE CIRCADIAN SLEEP-WAKE CYCLE

FINAL TECHNICAL REPORT

APRIL 1, 1983 - MARCH 31, 1986

Submitted By

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I. RESEARCH OBJECTIVES

This research program was designed to develop new pharmacological techniques to reset the timing of the circadian (approximately 24-hour) sleep-wake cycle so that an individual could be fully awake at any predetermined point in the 24-hour day. This capability would be of direct benefit in minimizing the deleterious effects of jet-lag and could promote alertness in facilities which must be staffed 24 hours a day.

The techniques involved the use of certain specific, non-toxic, pharmacologic agents which reset the timing of those circadian pacemakers in the hypothalamus which generate the natural sleep-wake cycle. This strategy would avoid the multiple problems associated with the current use of stimulants and hypnotics which, it is now well known, fail to induce normal wakefulness or sleep. A diurnal primate, the squirrel monkey (<u>Saimiri sciureus</u>), was used for these studies; the circadian neurophysiology and sleep-wake physiology of this species were defined under previous AFOSR support. This animal has a consolidated sleep-wake cycle comparable to that of humans. In addition, a rodent chronopharmacological screening capability was to be established and used to identify potential phase-resetting agents. The research described aimed to characterize and test the phase-resetting of phase related agents, as well as to explore other approaches to circadian phase resetting.

The specific aims included:

A. Examination of the phase resetting effects of anisomycin on the squirrel monkey circadian system.

B. Quantification of the rates of resynchronization after abrupt zeitgeber shifts.

C. Identify other potential phase resetting agents and determine their effect on the circadian sleep-wake cycle.

D. Assay the neural activity of circadian pacemakers after pharmacological injections of phase resetting agents.

E. Develop a method to reset circadian pacemakers using environmental lighting.

II. RESEARCH ACCOMPLISHMENTS

During the three-year period of support. we utilized our prior research
on the anatomy and physiology of the circadian timing system to characterize
the control of the sleep wake cycle in the squirrel monkey and to start to
develop the strategies for enhancing phase control using pharmacological
agents   This latter aim required the development of a rodent
chronopharmacological screening facility in addition to our primate research
facilities. The research accomplishments during the three-year period are
summarized below.

A. Circadian Sleep-Wake Organization in Squirrel Monkeys

We first analyzed the circadian sleep-wake and temperature rhythms of the
squirrel monkey in entrained and free-running conditions and characterized
their waveforms and phase relationships. Four adult male squirrel monkeys
weighing 900g were studied in LD 12:12 for one week and then in LL for 10-14
days.

In entrained conditions (LD), the sleep stages tended to be consolidated
into the hours of darkness. Gross locomotor activity corresponded well with
polygraphic wakefulness. A sharp downslope associated with the onset of sleep
was seen in the temperature waveform. Wakefulness, at the beginning of the
subjective day, was associated with the sharp rising edge of the temperature
curve. Least-squares analysis of wakefulness and temperature data indicated
the group mean period of the fundamental components was not statistically
different from 24.0 h.

All animals developed a circadian period of >24.0 h in free-running
conditions (LL). The fundamental components for temperature and wakefulness
were 24.69 ± 0.12 and 24.75 ± 0.18 h, respectively. In paired-t analysis.
the mean increase in period from LD to LL was 0.57 h (p<0.005) for temperature
and 0.69 h (p<0.01) for wakefulness. In LL subjective night onset was
associated with the downslope of the temperature curve. and sleep began later
than in LD. The circadian phases of sleep onset for each animal in LD were
similar, with a mean of 170°. In LL the distribution of sleep onsets was
centered near the middescending portion of the temperature curve, with a mean
of 187° and a wider range ($171-200^{\circ}$).

B. Resynchronization After Phase Shifts
of the LD Cycle

We completed our studies of the responses of the circadian sleep-wake
cycle to 8-hour phase advances and 8-hour phase delays of the light-dark (LD
12:12) cycle. Four squirrel monkeys (Saimiri sciureus), each weighing
approximately 900g, were surgically implanted, under general anesthesia, with
electrodes for recording fronto-central EEG, EOG and dorsal neck EMG. a cranial
thermistor, and a small mercury switch to record locomotor activity. The
animals were separately housed in chambers which maintained a 12-hr 60
lux:12-hr 0 lux light-dark (L:D) cycle. Daily care was given only during the
60 lux intervals. Food and water were available ad libitum.

After a post-operative recovery of at least one month and entrainment to LD
12:12. the animals entered a protocol of 2-3 days baseline. 8 hour phase shift

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(2 animals delay shift first, 2 animals advance shift first) and 7-10 days of post-shift recordings with follow up recordings at two weeks. Then each animal was studied again using the other direction of phase shift. The delay shift was accomplished by extending the interval of light by 8 hours, and the advance shift was obtained by shortening the light interval by 8 hours.

Locomotor activity and cranial temperature were automatically recorded every 15 minutes. The polygraphic record ran continuously and was subsequently hand-scored in one-minute segments using the following stages: AWAKE, TRANSITIONAL. NON-REM, REM. The scoring criteria and other technical details are reported elsewhere.

When compared by best-fitting asymptotic exponentials, resynchronization following an 8-hour advance shift required longer than that after an 8-hour delay phase shift. The mean times for 90% resynchronization of temperature acrophase was 4.1 ± 0.5 days after delay shift, and 5.2 ± 0.5 days following advance shift (difference 1.1 day. paired-t $p<0.05$). The resynchronization rates of activity acrophases showed a greater difference, with mean 90% resynchronization times of 2.9 ± 0.9 days for the delay shift and 5.8 ± 1.2 days for the advance shift (difference 2.9, paired-t $p<0.05$). There was no significant difference between temperature and activity acrophase resynchronization times for either direction of phase shift when the first post-shift acrophase was omitted from the curve-fitting. With the first post-shift acrophase included, activity appeared to resynchronize more quickly than temperature for the delay phase shift.

The reconsolidation of sleep stages into the phase shifted circadian interval of darkness occurred smoothly over two days following the delay phase shift. However, full phase resetting of the central circadian measures of non-REM and REM sleep required four to five days. For the 8-hour phase advance shift, five days were required for the sleep to reconsolidate into the subjective night. The circadian central measure of REM sleep resynchronized in about five days, and the central measure of non-REM sleep was reset over five to seven days.

C. Pharmacological Resetting of the Circadian System of the Squirrel Monkey

Three agents have been investigated using the squirrel monkey system. The first (anisomycin) proved to have no net phase shifting effects when compared to saline controls. The second. sodium valproate, caused alterations in the circadian pacemaker period and the third, muramyl dipeptide, induced sleep without altering circadian phase.

(1.) Anisomycin

Initial pilot studies using intraventricular injection of 100 micrograms of anisomycin via implanted intraventricular guide tubes induced phase shifts in free-running squirrel monkeys, but subsequent injections of saline vehicle were shown to induce comparable shifts (an important but not always conducted control for the pharmacology of the circadian system). A total of 8 squirrel monkeys were studied with 23 injections at monthly intervals in animals maintained in free-running conditions. Nine saline injection controls were also given as part of the protocol. The mechanism by which intraventricular

injection of saline vehicle caused a phase shift is not clear.

(2.) Sodium Valproate

We have undertaken a series of investigations on the effects of the antiepileptic drug, sodium valproate, on the circadian rhythms of activity and temperature in the squirrel monkey. This agent enhances gamma-aminobutyric acid (GABA) levels in the brain by a number of mechanisms which enhance synthesis and decrease breakdown. GABA is an inhibitory amino acid found throughout the brain but in high concentrations in the hypothalamus. Within the hypothalamus the suprachiasmatic nucleus contains the highest concentration of glutamate dehydrogenase, the enzyme responsible for the synthesis of GABA from glutamic acid.

The daily administration of valproate to 12 free-running squirrel monkeys maintained in constant illumination produced either a shortening or a lengthening of both the temperature and activity rhythms in individual animals. For example, in one study 3 animals showed a shortening, and 3 animals showed a lengthening of these rhythms (Fig. 1). In animals placed on the same experimental protocol for the second time, valproate reproduced the shortening or lengthening in individual animals.

The fact that this effect may be exerted at the level of the suprachiasmatic nucleus is supported by the fact that injection of valproate intraperitoneally in squirrel monkeys produced a decrease in neural multiple unit activity recorded from the hypothalamus in free-ranging animals. Corroboratory evidence was obtained from slices of rat brain containing the SCN, to which applications of GABA over a 24-hour period produced an inhibition of spontaneous firing (electrical activity) in the SCN, which seems to be phase dependent with a reduction in activity being most marked during the subjective day.

(3.) Muramyl Dipeptide

The third substance, a synthetic analog of sleep factor S, produced an interesting result in that it induced sleep but did not cause any lasting phase resetting of the circadian system.

The circadian sleep-wake and body temperature cycles of squirrel monkeys were monitored continuously in an environment free of time cues before and after a 50-nmol injection of a synthetic muramyl dipeptide (MDP) either one hour after wake-up time (subjective day) or just before sleep time (subjective night). At both phases decreases in percent time awake (relative to saline controls) were observed. After administration of MDP early in the subjective day, the animals exhibited alert wakefulness only 47.4% of the daytime, compared with a mean 86.7% of daytime after a saline control injection (Fig. 2). The transitional stage was significantly elevated from 6.2 to 28.7% of time after MDP. Non-rapid-eye-movement (NREM) sleep was elevated in 4 of 5 animals, but in the fifth animal, which had the least consolidated baseline sleep-wake pattern, a small decrease in non-REM sleep was seen. Regression analysis demonstrated a significant ($p < 0.01$) relationship between the degree of sleep-wake consolidation and the effect of MDP on non-REM sleep. Normal sleep behaviors and spontaneous arousals were observed. MDP given at the circadian nighttime of two animals resulted in sleep and transitional episodes
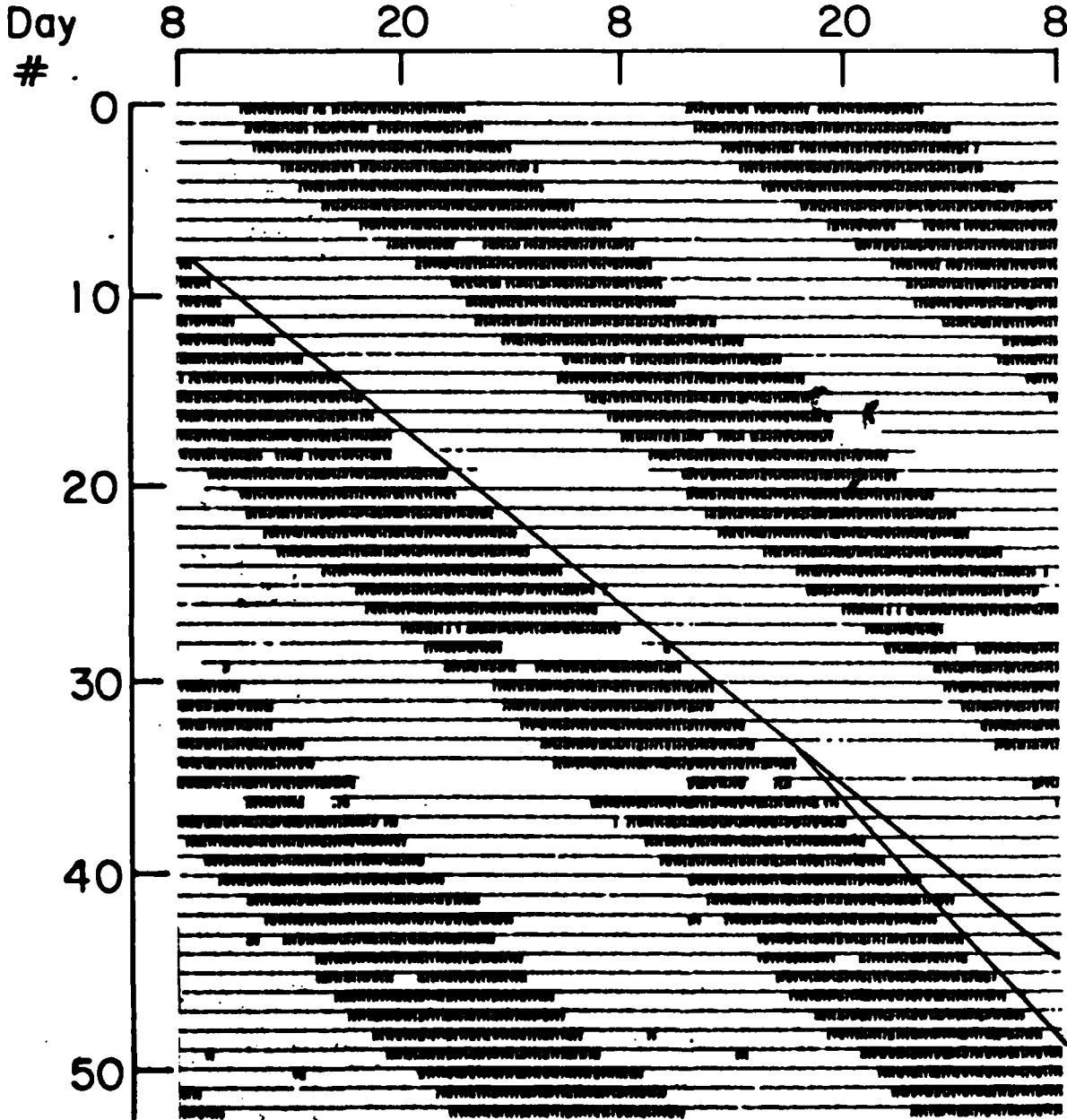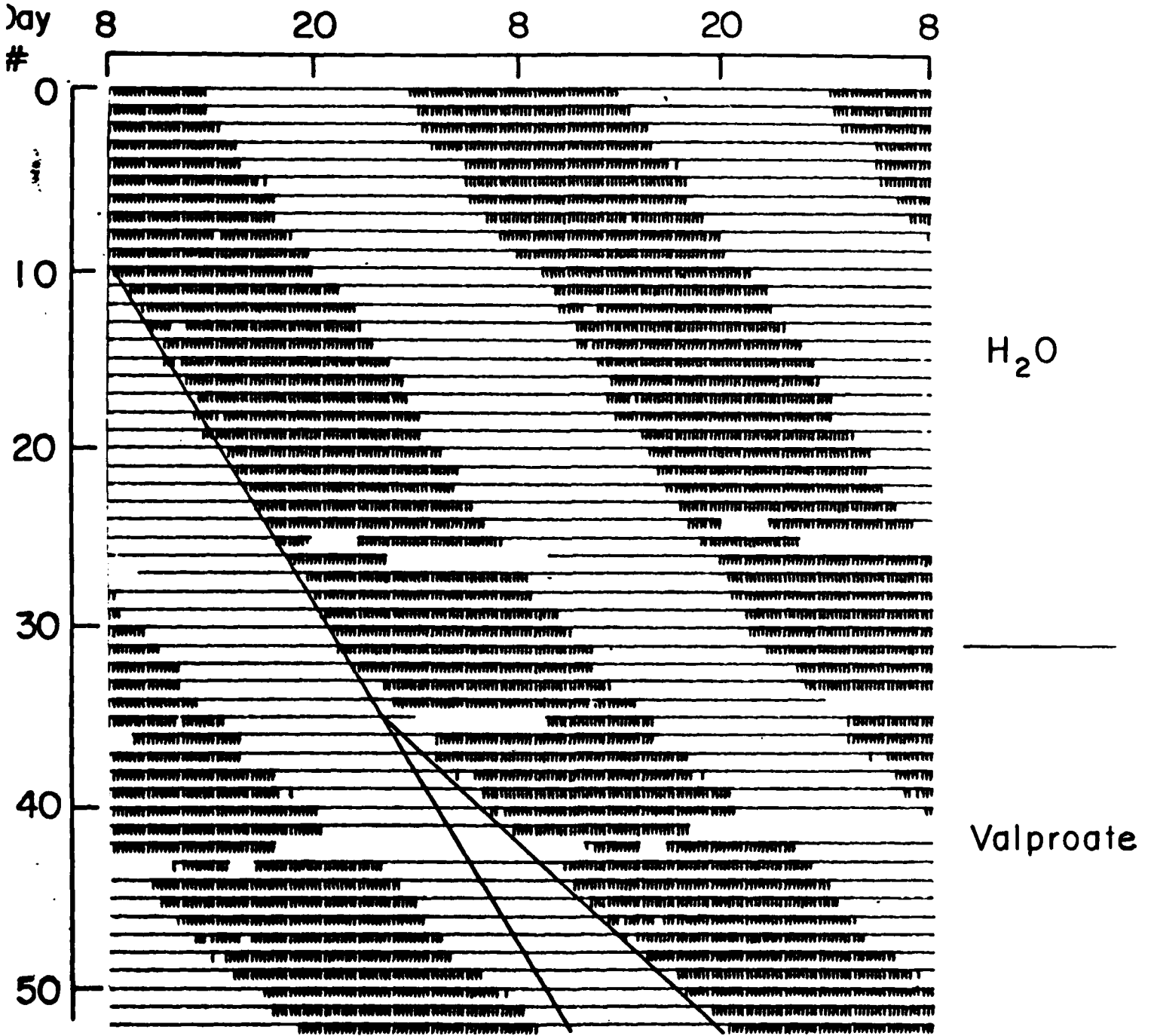
TEMPERATURE   T910B0

Time of Day (hrs)

FIGURE 1A, AND .....

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Time of Day (hrs)

FIGURE 1B: Body temperature above mean plotted by vertical pen strokes in raster format double plot showing free-running rhythm which was either shortened (left) or lengthened with administration of sodium valproate depending on the animal treated.
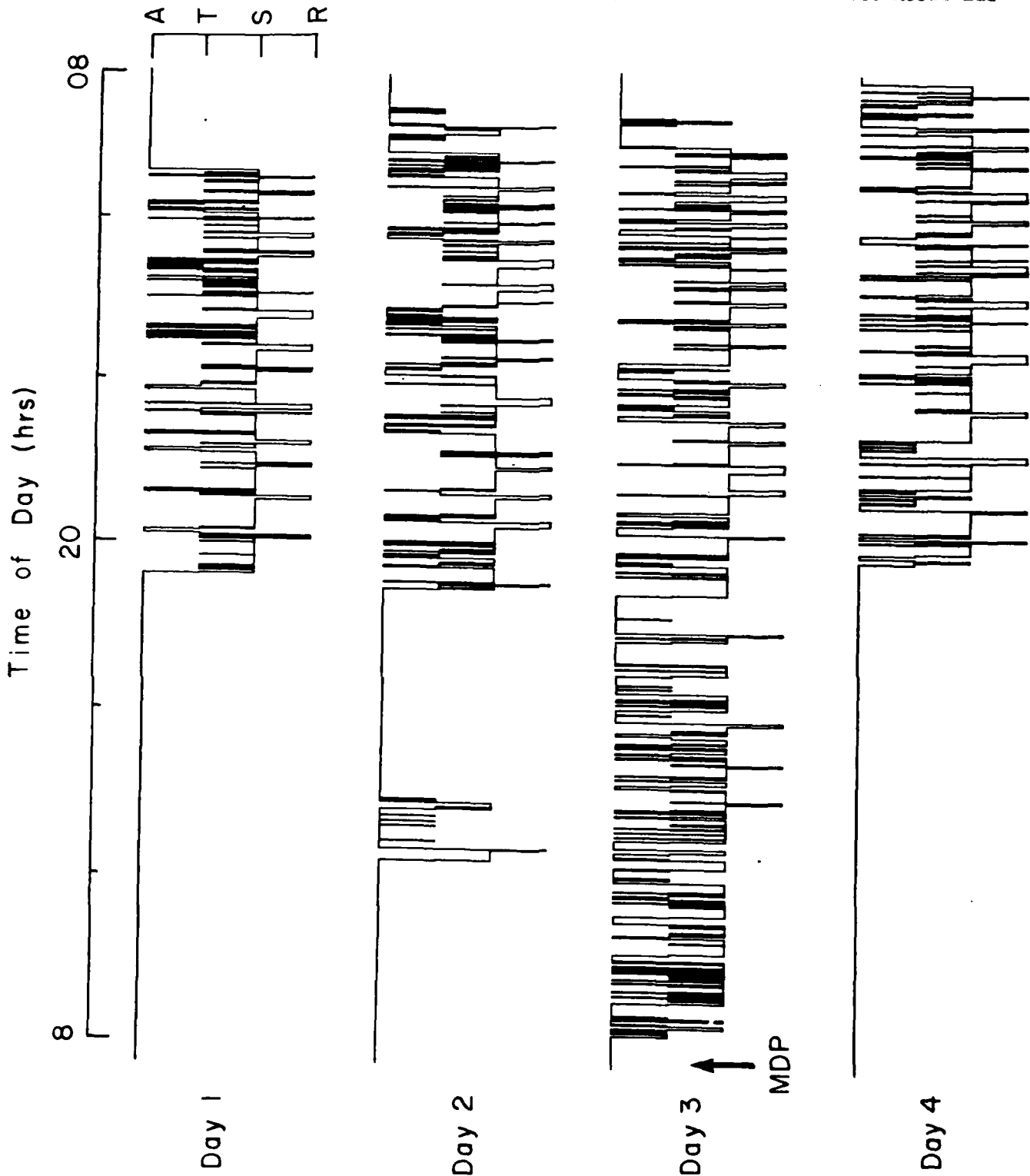
FIGURE 2 Sleep-wake stages (A, awake; T, transitional sleep; S, non-rapid-eye-movement sleep; R, rapid-eye-movement sleep) from squirrel monkey in continuous light show consolidated sleep-wake cycle on baseline days (days 1 and 2). After muramyl dipeptide (MDP) was given on day 3 (arrow), transitional and sleep episodes with numerous arousals occurred throughout subjective day. Four of 5 monkeys had similar responses. Sleep during following subjective night was similar to non-injection baseline pattern. Composition and structure of sleep and wake stages on day 4 appear no different from pattern on baseline days.

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occupying 84% of the subjective night vs. 73% of time asleep after control injection. Again, these were mostly transitional and non-REM sleep stages, and they persisted 4-5 hours into the predicted circadian daytime. REM sleep appeared to be suppressed in the early hours after injection at either time of day. Body temperature was elevated to about $2^{\circ}C$ above the 24-hour mean level at both injection times, and the effects lasted about 10 hours. Despite the marked influences of MDP on the sleep-wake pattern and body temperature rhythm over the 24 hours after administration, the circadian timing system demonstrated no consistent shifts in phase (Fig. 3). MDP in squirrel monkeys appears to modify sleep-wake states by mechanisms that do not require phase resetting the circadian timing system.

D. Physiological Correlates of Sleep and Effects of Sleep Deprivation

We have initiated a new series of experiments in squirrel monkeys chronically prepared to allow concurrent monitoring of cortical and subcortical EEG, EMG, EOG, MUA from one or more brain sites, and body temperature and motor activity. MUA, temperature and activity are recorded continuously (at 5, 15 and 30 min intervals, respectively), while EEG, EMG and EOG are recorded for 1-4 day intervals on a polygraph and on FM tape for later power spectral analysis. All EEG and MUA recording electrodes (the same subcortical electrodes are used to record MUA and EEG from such structures as the LGN, hippocampus and septum) are attached to preamplifiers that are cemented to the skull, a procedure which has the important advantage of minimizing movement artifacts and thus providing good EEG records during both sleep and wakefulness.

Concurrent sleep and MUA recordings have been obtained in three monkeys that were maintained under LD 12:12, followed by LL (60 lux). Figure 4 shows MUA patterns and polygraphically determined sleep states in 2 monkeys under LD (lights on from 0800 to 2000 h). The first (right panels) shows consolidated nocturnal sleep with only a few brief awakenings at the beginning and end of the night, as well as three polygraphically confirmed daytime naps. The naps were accompanied by sharp decreases in MUA in both the LGN and the hippocampus, while nighttime awakenings and REM episodes were accompanied by increases in MUA that were more pronounced in the hippocampal record. The second monkey (left panels) had frequent brief awakenings at night and no daytime naps. The nocturnal awakenings and REM episodes were also accompanied by increases in MUA, but only in the septum and not the LGN. The MUA records obtained from the same monkey but without concurrent sleep recordings, suggest that LGN activity increases during nighttime awakenings when the monkey is under LL, where septal MUA increases during awakenings and REM episodes regardless of the lighting conditions.

The difference in the MUA patterns recorded from these two LGN placements suggests different locations of the electrode tips within that nucleus (histology on these animals is not yet available). This is supported by the fact that the light-evoked potentials obtained from these two electrodes during surgery were also different, as were the EEG patterns recorded during different sleep states. A relationship between the shape of the light-evoked potentials and the EEG patterns (particularly the presence of PGO waves during REM sleep) recorded from different locations within the LGN has been documented in the cat (Davis, 1976).
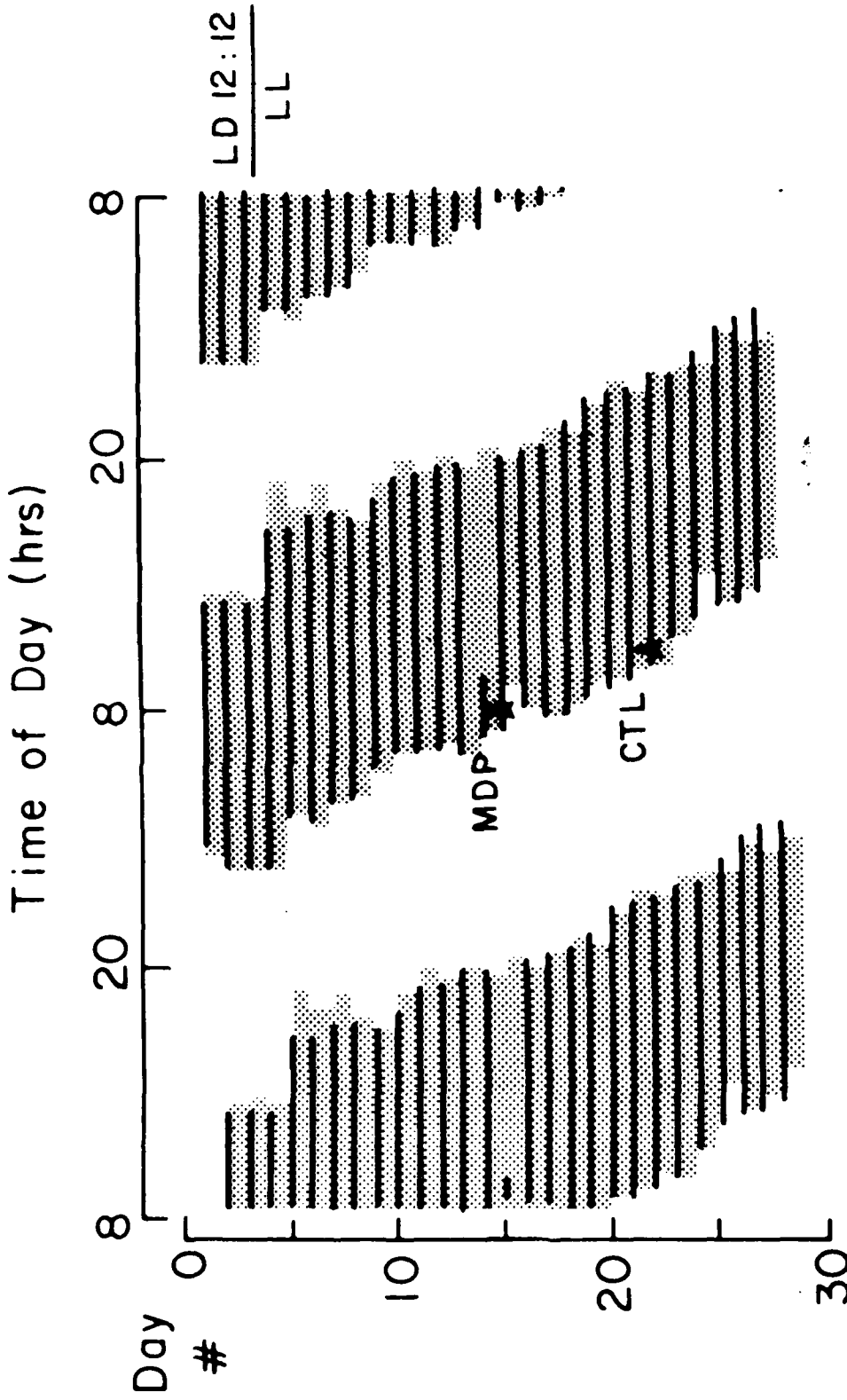
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FIGURE 3   Temperature above mean (stippling) and locomotor activity (black bar) co-plotted in double raster format. On day 4, animal was released from light-dark (LD) schedule (lights-on 1200-2400 h) into free-running conditions (LL). Muramyl dipeptide (MDP) given early in subjective day produced reduction in activity counts for remainder of subjective day. Neither MDP nor saline control injection (CTL) appeared to shift phase of either free-running rhythm.

We have also obtained preliminary data from monkeys that were sleep-deprived on different occasions while in LL. Sleep deprivation lasted for 7, 13, 21, and 25 hours and up to 40 hours, starting one hour before the expected time of sleep onset. Sleep states and cortical EEG power density were determined from continuous polygraphic and tape recordings starting 24 hours before the start of each sleep deprivation session and continuing through the first two recovery 'nights' (i.e. subjective nights). Figure 4 shows the results of a 13-hour sleep deprivation session, which ended at the beginning of subjective day. Despite the sleep deprivation, the animal remained awake for about an hour after being released, then showed several short sleep episodes interrupted by bouts of sustained wakefulness before beginning a consolidated sleep episode at the time predicted from its pre-deprivation free-running rhythms. Neither this nor the 7-hour and 21-hour sleep deprivation sessions caused any change in the timing of the animal's main sleep episode, nor did they cause a phase shift of the free-running temperature and activity rhythms (Fig. 6). Figure 5 also shows EEG power in the 0-25 hz frequency band for that sleep deprivation session (a plot of EEG power in the 0.5-4 hz frequency band gave essentially similar results with only a small decrease in amplitude, indicating that most of the EEG power is accounted for by activity in the delta range). A correlation between the two graphs of Figure 5 is evident, with a clear increase in EEG power during each episode of NREM sleep, and decreases during episodes of wakefulness and of REM sleep. The size of the NREM peaks also shows a progressive decrease during the baseline night and the first recovery night. The EEG power graph shows only a small increase in power in the first recovery night relative to the baseline night, suggesting that some of the effects of sleep deprivation must have been dissipated during the 'daytime' sleep episodes. One interesting aspect of these data is the fact that the EEG power during NREM sleep episodes was greater at the beginning of the first recovery night than during the daytime sleep episodes that immediately followed sleep deprivation, despite the fact that the latter were preceded by a much longer period of wakefulness than the former. Confirmation of this result in additional monkeys would represent strong evidence for a circadian modulation of EEG power.

Finally, we have been exploring the possibility of recording EEG theta activity from subcortical electrodes, as an additional index of REM sleep. Several researchers have commented on the difficulty of recording theta activity from the primate hippocampus (cf. Robinson, 1980), but more work remains to be done before accepting the conclusion that this pattern is entirely absent in primates. Thus far, we have recorded hippocampal EEG in five squirrel monkeys and have also been unable to find any visually identifiable theta activity during REM sleep. An attempt to elicit hippocampal theta (in acute experiments) by administration of physostigmine in three monkeys was equally ineffective, but a recognizable theta pattern was obtained in two other monkeys from the medial septal nucleus and the vertical limb of the diagonal band. We have also obtained some evidence for the presence of theta during REM sleep by quantitative analysis of taped hippocampal EEG. The analysis, performed by Dr. J.R. Pappenheimer, consists of obtaining the ratio of EEG amplitude in the 4-8 hz frequency range over that in the 1-4 hz range (Pappenheimer, 1984). The results were in perfect agreement with our own scoring of sleep states, showing clear increases in the amplitude ratio during each REM sleep episode. We are now in the process of anlayzing other taped samples of hippocampal and septal EEG to determine

FIG. 4 Concurrently recorded MUA patterns from two brain regions and polygraphically determined sleep states in two monkeys under LD 12:12 (lights-on: 0800-2000 h). The septal and hippocampal records show clear increases in MUA at night coinciding with episodes of wakefulness and of REM sleep. Similar but smaller changes are also visible in one LGN record (right). The two MUA records on the right also show sharp decreases in MUA coinciding with daytime naps.
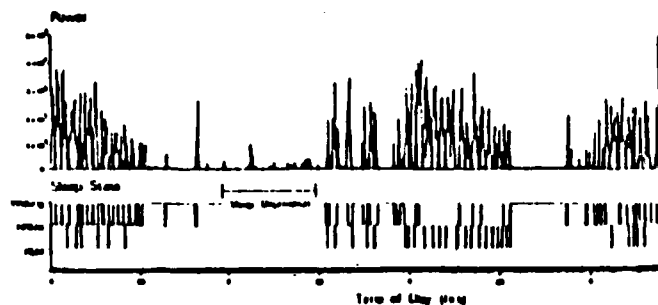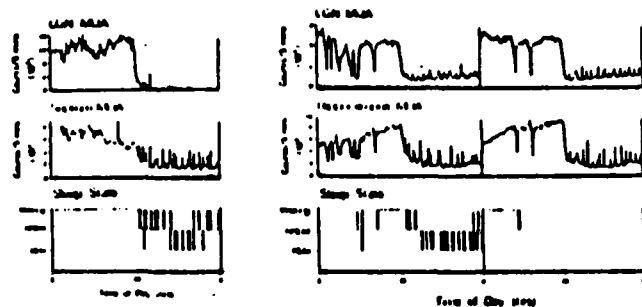
FIG. 5 EEG power density in the 0-25 hz frequency range (1-min averages calculated from 12 consecutive 5 sec segments) and polygraphically determined sleep states (in 1-min epochs) from a monkey that was sleep-deprived for 13 h in LL, starting 1 h before expected sleep onset. Peaks in EEG power occurred during each episode of NREM sleep, with a decrease in amplitude over the course of the baseline and first recovery "nights" (the second recovery night is not included in its entirety in these records). See text for other explanations.

FIG. 6 Portion of the activity record of a monkey that was sleep-deprived for 21 h while in LL. Sleep deprivation began 1 h before expected sleep onset and was accompanied by the sustained increase in activity visible on days 24 and 25 of the record. The main consolidated sleep episode of the first recovery "night" began at the predicted time, despite the fact that sleep deprivation had ended 5 h earlier. That sleep episode lasted somewhat longer than usual, but no permanent phase shift of the free-running rhythm was observed.

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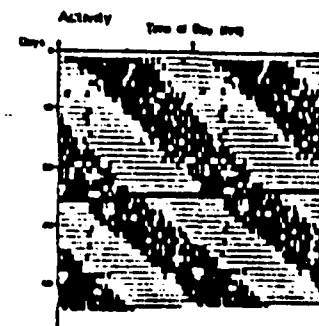
whether EEG power density in the theta range also increases during REM sleep, and whether it shows circadian variation, as was recently reported for EEG theta activity in the mouse (Welsh et al., 1984).

E. Entrainment by LD Cycles with 3-h Dark Segments

This study was designed to examine the resetting of circadian rhythms by 3-h dark pulses in squirrel monkeys maintained in LL. Instead of measuring phase shifts induced by single dark pulses, however, we exposed monkeys to LD cycles with 3-h dark segments, while systematically varying the intensity (60 vs. 600 lux) and the duration of the light segment, such that the period of the LD cycles ranged between 23.0 h and 27.0 h. This procedure allowed us (a) to find out whether these LD cycles are capable of entraining circadian rhythms and to determine the limits of such entrainment (i.e. the shortest and longest LD cycles that will produce entrainment), (b) to compare the resetting effects of darkness with those of light, and (c) to attempt to produce internal desynchronization between the circadian rhythms of body temperature and of rest-activity.

Circadian rhythms of body temperature and motor activity were continuously recorded for up to 9 months from 5 squirrel monkeys individually maintained in isolation chambers. All were initially kept under LL (60 lux). Three monkeys were then exposed to LD cycles with a 3-h dark segment and with a period (T) shorter than that of their free-running rhythms (τ approximately 25 hours), while the remaining two were exposed to LD cycles with T longer than τ.

Figure 7 shows complete phase plots of the temperature and activity rhythms of 2 monkeys, representing the time of the daily peak of each of the rhythms as a function of days in the experiment. One monkey (left) was first exposed to a 24-h cycle (LD 21:3) with light intensity set at 60 lux. The LD cycle produced relative coordination (i.e. a slowing down and speeding up of the rhythms depending on their phase relative to dark onset) but failed to entrain the rhythms. Entrainment did occur, however, when light intensity was increased to 600 lux. Relative coordination was obtained again when the period of the LD cycle was shortened to 23.5 h. The second monkey (right) was entrained to a 26-h cycle at 60 lux, but not to a 27 h or 26.5 h cycle. Entrainment to a 26.5 h cycle was obtained at 600 lux, and relative coordination under a 27-h cycle. Towards the end of this latter condition (Days 238-245), internal desynchronization occurred between the temperature rhythm, which remained synchronized to the LD cycle, and the activity rhythm, which broke loose and free-ran at a shorter period.

In summary, the results indicate that LD cycles with 3-h dark segments can entrain circadian rhythms in the squirrel monkey and that such entrainment is possible at periods both shorter and longer than those of the free-running rhythms, indicating that 3-h dark pulses can phase-advance as well as phase-delay the rhythms. Entrainment to short LD cycles occurred with the dark segment falling early in the monkey's subjective night, while entrainment to long LD cycles was obtained when the dark segment fell during late subjective night and early subjective day. This indicates that dark pulses cause phase shifts in a direction opposite to that of phase shifts caused by light pulses and, therefore, that the PRCs for light and darkness are mirror images.
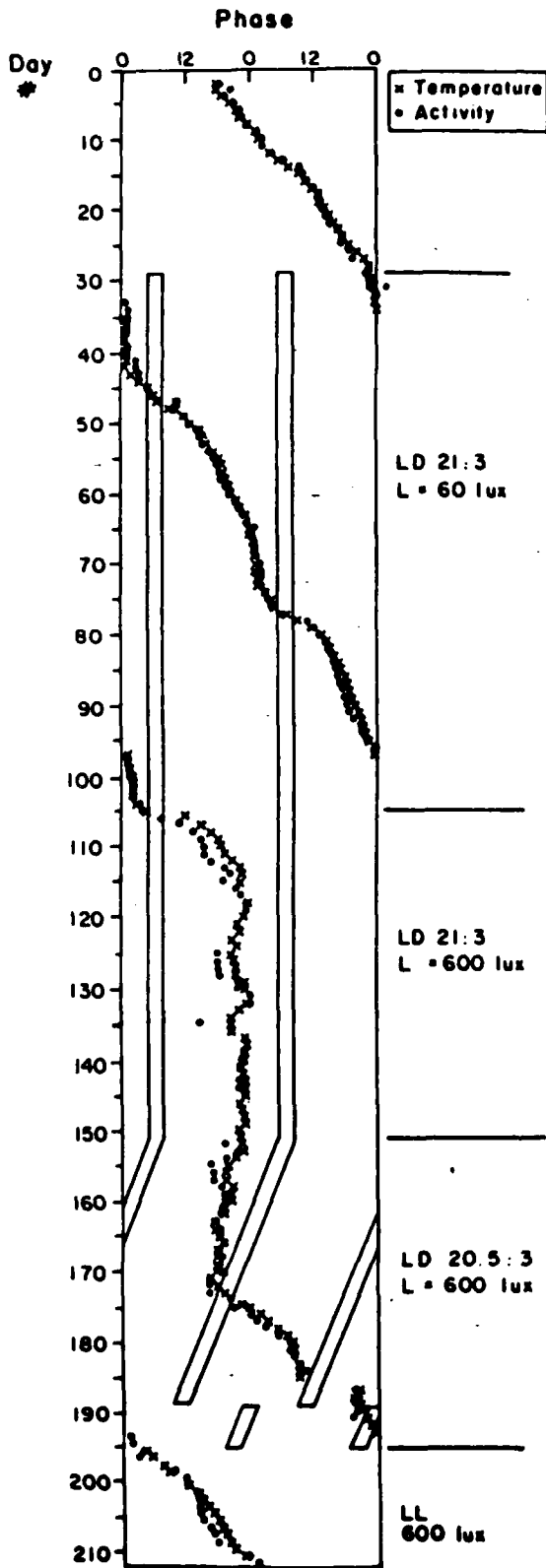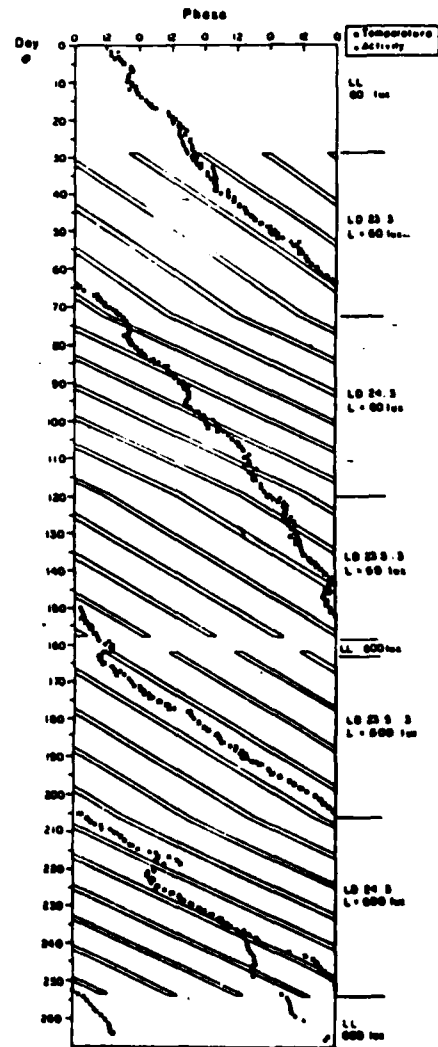
Phase

FIG. 7

Phase plot of temperature (X) and activity (O) rhythms in animals exposed to LD 3:21 cycles.

The limits of entrainment at 60 lux were found to lie between 24.0 h and 26.0 h. These limits were extended by 30-60 min in either direction by increasing light intensity to 600 lux. Thus, the size of the phase shifts caused by dark pulses is proportional to the size of the intensity step from light to darkness.

Finally, the results suggest that such LD cycles can induce internal desynchronization in monkeys, without having as much of a masking effect as LD cycles with longer dark segments.

F. Rodent Chronopharmacological Facility

The rodent temporal isolation facility has been assembled in a room in the 6th floor animal research facilities. For the initial studies we have selected the Syrian hamster (Mesocricetus auratus) and have verified the reliability of the wheel-running and recording systems. We have also conducted a number of pharmacological studies (see below) using rats. Temporal isolation appears to be satisfactory with reliable free-running records obtained. Each chamber is divided into 4 stacked, horizontal rows; each row holds 3 cages, each of which contains one 100g male Syrian hamster. Each row's lighting can be separately controlled. Activity is recorded as wheel-running behavior. Experiments have demonstrated that one animal's activity does not affect that of others in the same row.

Each 180° wheel-turn activates a microswitch and is recorded on an Esterline Angus chart recorder. The electrical signals are interfaced with an Apple IIe computer for data analysis of period length, phase and other circadian parameters.

G. Benzodiazepine Receptor Rhythms

Male Sprague-Dawley rats (300-400g, Charles River Breeding) were maintained for 4 weeks (January-February) on a photoperiodic cycle with 14 hours of light (05h00-19h00) and 10 hours of dark (19h00-05h00) and allowed free access to water and lab chow. At 6-hour intervals over a 36-hour period, groups of rats were killed by decapitation, the whole brains rapidly removed onto dry ice chilled coverslips and dissected. Frontal lobes, temporoparietal lobes, hypothalamus, cerebellum and medulla/pons were frozen at -70°C and stored until use (5-11 days). Washed synaptic plasma membranes prepared from these regions were incubated with ^{3}H-flunitrazepam (New England Nuclear Corp., Boston, MA) at 5 concentrations in the range 0.5-7.5nM in the absence or presence of one micromolar unlabeled flurazepam. Total receptor number (Bmax) and affinity (Kd) were determined by Scatchard analysis of the saturation isotherms of specific ^{3}H-flunitrazepam binding. Membrane protein concentrations were determined by the method of Lowry et al.

Prominent daily rhythms in benzodiazepine receptor number (Bmax) were observed in the frontal lobe and cerebellum but not in the other regions examined (Fig. 8). In the frontal lobe, binding was highest at 06h00 and 12h00, corresponding to the period of sleep/low activity (lights on) in the rat. A significant (24-31 percent) decrease in receptor number was noted at 18h00 in anticipation of waking (lights off at 19h00). At 24h00 Bmax was intermediate to the values at 18h00 and 06h00 but not statistically different from the 06h00 level.

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Receptor binding in the cerebellum was maximal at 12h00, in the middle of the sleep/low activity period, and lowest at 18h00 and 06h00. Again, a significant (29-42 percent) reduction in receptor number occurred from 12h00 to 18h00, anticipating lights off at 19h00. No significant daily fluctuations in receptor affinity were observed.

Our data demonstrate that normal rat brain benzodiazepine receptors undergo rapid fluctuations in number during the course of a day with 24-42 percent change in Bmax occurring over a 6-hour period. This implies that the receptors, or at least a subpopulation of them, have a half-life in the membrane in the order of a few hours. Further, if our results are generally applicable, studies of receptor binding in pathological post-mortem brains should consider the time-dependent fluctuations in binding which occur normally. This may necessitate time-of-death controls.

H. Suprachiasmatic Regulation of Benzodiazepine and GABA Receptor Rhythms

The circadian variation in total receptor number (Bmax) was determined for ^{3}H-flunitrazepam and ^{3}H-muscimol binding in male Sprague-Dawley rats maintained in an LD 14:10 light-dark cycle. The receptor assays were conducted in control animals and in animals which 3 weeks previously had had complete SCN lesions placed (verified by subsequent histology). Figure 9 shows that the SCN lesions eliminated the rhythms seen in frontal areas of the brain in both benzodiazepine and GABA receptors.
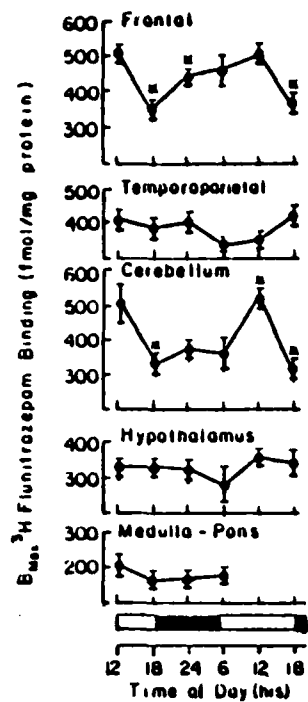
FIG.8 Daily rhythms of benzodiazepine receptor binding in regions of rat brain. Values for Bmax (fmol/mg protein) were determined by Scatchard analysis of saturation isotherms of the specific binding of ^{3}H-flunitrazepam (0.5-7.5nM) to washed synaptic plasma membranes. On the horizontal axis light bars represent periods of sleep/low activity (lights on) and dark bars represent wake/high activity (lights off). Groups contained 4-5 rats and each point represents mean ± S.E.M. (n=3).

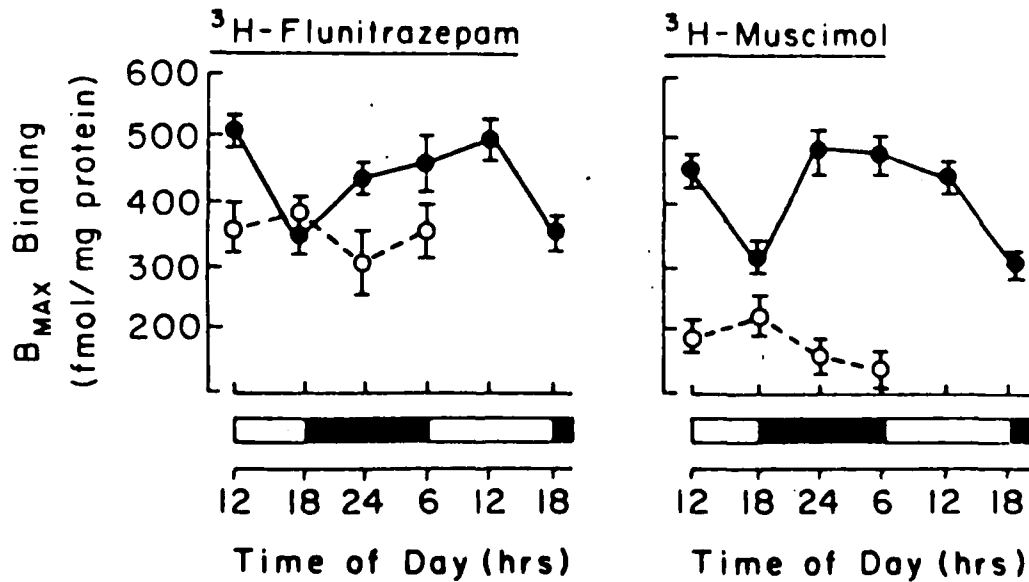*p<0.001, significantly different from preceding point, Student t-test.

FIG. 9 Daily rhythm of benzodiazepine (left) and GABA (right) receptor binding in intact (solid line) and SCN-lesioned (dashed line) rats.

III.   CUMULATIVE CHRONOLOGICAL LIST OF WRITTEN
       PUBLICATIONS IN TECHNICAL JOURNALS

The three-year funding period (1983 - 1986) resulted in over 20 scientific articles and published abstracts.

Six copies of each of the following manuscripts and published articles were forwarded to the Air Force Office of Scientific Research on March 24, 1986, in advance of submission of this Final Technical Report:

MANUSCRIPTS SUBMITTED FOR PUBLICATION

Moore-Ede, M.C.
Physiology of the circadian timing system: Predictive versus Reactive Homeostasis. 30th Annual Bowditch Lecture.
American Journal of Physiology, In Press, 1986.

Wexler, D.B., Moore-Ede, M.C.
Resynchronization of circadian sleep-wake and temperature cycles in the squirrel monkey following phase shifts of the environmental light-dark cycle.
Aviation Space and Environmental Medicine, In Press, 1986.

REPRINTS OF PUBLICATIONS

Kronauer, R.E., Czeisler, C.A., Pilato, S.F., Moore-Ede, M.C., Weitzman, E.D.
Mathematical representation of the human circadian system: Two interacting oscillators which affect sleep.
Chase, M., ed. *Sleep Disorders: Basic and Clinical Research*. New York: Spectrum Publications, Inc., 1983, pp. 173-194.

Moore-Ede, M.C.
Hypothermia: A timing disorder of circadian thermoregulatory rhythms?
Pozos, R.S., Wittmers, L.E., eds. *The Nature and Treatment of Hypothermia*, *Vol. 2*. Minneapolis: University of Minnesota Press, 1983, pp. 69-80.

Moore-Ede, M.C.
The circadian timing system in mammals: Two pacemakers preside over many secondary oscillators.
Fed. Proc. 42 (11): 2802-2808, 1983.

Weitzman, E.D., Czeisler, C.A., Zimmerman, J.C., Moore-Ede, M.C., Ronda, J.M.
Biological rhythms in man: Internal physiological organization during non-entrained (free-running) conditions and application to delayed sleep phase syndrome.
Chase, M., ed. *Sleep Disorders: Basic and Clinical Research*. New York: Spectrum Publications, Inc., 1983, pp. 153-171.

Fuller, C.A., Lydic, R., Sulzman, F.M., Albers, H.E., Tepper, B., Moore-Ede, M.C.
Auditory entrainment of primate drinking rhythms following partial suprachiasmatic nuclei lesions.
Physiol. Behav. 31 (4): 573-576, 1983.

Schwartz, W.J., Reppert, S.M., Eagan, S.M., Moore-Ede, M.C.
In vivo metabolic activity of the suprachiasmatic nuclei: A comparative study.
Brain Research 274: 184-187, 1983.

Moore-Ede, M.C., Czeisler, C.A., Richardson, G.S.
Circadian timekeeping health and disease. Part 1: Basic properties of circadian pacemakers.
New England Journal of Medicine 309 (8): 469-476, 1983.
Part 2: Clinical implications of circadian rhythmicity.
New England Journal of Medicine 309 (9): 530-536, 1983.

Gander, P.H., Moore-Ede, M.C.
Light-dark masking of circadian temperature and activity rhythms in squirrel monkeys.
American Journal of Physiology 245 (14): R927-R934, 1983.

Czeisler, C.A., Moore-Ede, M.C., Coleman, R.M.
Resetting circadian clocks: Applications to sleep disorders medicine and occupational health.
Guilleminault, C., Lugaresi, E., eds. *Sleep/Wake Disorders: Natural History, Epidemiology, and Long-Term Evolution.* New York: Raven Press, 1983, pp. 243-260.

Albers, H.E., Lydic, R., Gander, P.H., Moore-Ede, M.C.
Role of the suprachiasmatic nuclei in the circadian timing system of the squirrel monkey. I: The generation of rhythmicity.
Brain Research 300: 275-284, 1984.

Albers, H.E., Lydic, R., Moore-Ede, M.C.
Role of the suprachiasmatic nuclei in the circadian timing system of the squirrel monkey. II: Light-dark cycle entrainment.
Brain Research 300: 285-293, 1984.

Gander, P.H., Kronauer, R.E., Czeisler, C.A., Moore-Ede, M.C.
Simulating the action of zeitgebers on a coupled two-oscillator model of the human circadian system.
American Journal of Physiology 247 (16): R418-R426, 1984.

Gander, P.H., Kronauer, R.E., Czeisler, C.A., Moore-Ede, M.C.
Modeling the action of zeitgebers on the human circadian system: Comparisons of simulations and data.
American Journal of Physiology 247 (16): R427-R444, 1984.

Wexler, D.B., Moore-Ede, M.C.
Effects of a muramyl dipeptide on the temperature and sleep-wake cycle of
the squirrel monkey.
American Journal of Physiology 247 *(16):* R672-R680, 1984.

Moore-Ede, M.C.
The body's inner clocks.
Elliott, J.P., Hantula, R., McGeveran, W.A., Jr., eds. In: *Health & Medical
Horizons 1984.* New York: Macmillan Educational Company, 1984, pp. 167-175.

Borsook, D., Moore-Ede, M.C., Hedberg, T., Richardson, G.S., Brennan,
M.J.W.
Gamma-aminobutyric acid and the neural basis of circadian timekeeping:
Implications for pathophysiology and psychopharmacotherapy of circadian
based disorders.
Reinberg, A., Smolensky, M., Labrecque, G., eds. *Annual Review of
Chronopharmacology, Vol. 1.* New York: Pergamon Press, 1984, pp. 53-56.

Richardson, G.S., Moore-Ede, M.C., Czeisler, C.A., Dement, W.C.
Circadian rhythms of sleep & wakefulness in mice: Analysis using long-term
automated recording of sleep.
American Journal of Physiology 248 *(17):* R320-R330, 1985.

Apelgren, K.N., Frim, D.M., Harling-Berg, C.J., Gander, P.H., Moore-Ede,
M.C.
Effectiveness of cyclic intragastric feeding as a circadian zeitgeber in
the squirrel monkey.
Physiol. Behav. 34 *(3):* 335-340, 1985.

Wexler, D.B., Moore-Ede, M.C.
Circadian sleep-wake cycle organization in squirrel monkeys,
American Journal of Physiology 248 *(17):* R353-R362, 1985.

Moore-Ede, M.C., Richardson, G.S.
Medical implications of shift-work.
Annual Review of Medicine 36: 607-617, 1985.

Erny, B.C., Wexler, D.B., Moore-Ede, M.C.
Sleep-wake stages during the subjective night of the squirrel monkey.
Physiol. Behav. 35 *(2):* 189-194, 1985.

Gander, P.H., Lydic, R., Albers, H.E., Moore-Ede, M.C.
Forced internal desynchronization between circadian temperature and activity
rhythms in squirrel monkeys.
American Journal of Physiology 248 *(17):* R567-R572, 1985.

Brennan, M.J.W., Volicer, L., Moore-Ede, M.C., Borsook, D.
Daily rhythms of benzodiazepine receptor numbers in frontal lobe and
cerebellum of the rat.
Life Sciences 36 *(24):* 2333-2337, 1985.

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Fuller, C.A., Sulzman, F.M., Moore-Ede, M.C.
Role of heat loss and heat production in generation of the circadian
temperature rhythm of the squirrel monkey.
Physiol. Behav. 34 (4): 543-546, 1985.

IV. LIST OF PROFESSIONAL PERSONNEL
ASSOCIATED WITH THE RESEARCH EFFORT

1. Martin C. Moore-Ede, Principal Investigator
2. Dr. Ziad A. Boulos
3. Dr. Philippa H. Gander
4. Mr. David B. Wexler
5. Dr. David Borsook
6. Dr. Gary S. Richardson
7. Mr. Diomedes Logothetis
8. Mr. David K. Welsh
9. Ms. Elizabeth Klerman
10. Mr. Thomas A. Houpt

V.   INTERACTIONS

Dr. Moore-Ede has had multiple interactions with scientific colleagues at professional meetings and also with various governmental offices, including the scientific meetings of the Air Force Office of Scientific Research. The other professional staff have presented papers at scientific meetings and are constantly in touch with colleagues at other universities and at governmental laboratories.

A list is included of the scientific presentations that were made.

Six (6) copies of each of the following abstracts have been sent to the Air Force Office of Scientific Research:

ABSTRACTS

(1.) Wexler, D.B., Harling, C.J. and Moore-Ede, M.C.
Muramyl dipeptide induces non-REM sleep in squirrel monkeys. Presented at the 12th Annual Meeting of the Society for Neuroscience, Minneapolis, Minnesota, November 1982.

(2.) Czeisler, C.A., Moore-Ede, M.C., and Weitzman, E.D. Endogenous gating of awakening in man underlies circadian variation in spontaneous sleep duration. *Sleep Research*, 1983.

(3.) Moore-Ede, M.C.
Circadian regulation of sleep and body temperature in primates. International Union of Physiological Sciences XXIXth International Congress, Sydney, Australia, August 1983.

(4.) Gander, P.H., Kronauer, R.E., Czeisler, C.A. and Moore-Ede, M.C.
Entraining a two oscillator model for the human circadian system. International Union of Physiological Sciences XXIXth International Congress, Sydney, Australia, August 1983.

(5.) Boulos, Z., Logothetis, D.E. and Moore-Ede, M.C.
Circadian rhythms of multiple unit activity from hypothalamic and other brain stem areas of the squirrel monkey. 13th Annual Meeting of the Society for Neuroscience, Boston, Massachusetts, November 1983.

(6.) Hedberg, T.G. and Moore-Ede, M.C.
Circadian rhythmicity in multiple-unit activity of rat hypothalamic slice. 13th Annual Meeting of the Society for Neuroscience, Boston, Massachusetts, November 1983.

(7.) Apelgren, K.N., Frim, D.M., Harling-Berg, C.J., Gander, P.H. and Moore-Ede, M.C.
The effect of cyclic intragastric feeding on circadian rhythms in the squirrel
monkey. 17th Annual Meeting of the Association for Academic Surgery, Syracuse
New York, November 1983.

(8.) Kronauer, R.E., Gander, P., Moore-Ede, M.C., Czeisler, C.A.
A two-oscillator model derived from free-running human circadian rhythms
accurately predicts range of zeitgeber entrainment. Presented at the 4th Intl.
Congress of Sleep Research, Bologna, Italy, July 1983.

(9.) Moore-Ede, M.C.
The twenty-four hour society: Conflicts between environmental and biological
time. Presented at the Second Annual International Symposium on Man and
His Environment in Health and Disease, Dallas, Texas, February 1984.

(10.) Krieger, G.R. and Moore-Ede, M.C.
Shiftworker performance after a program of chronohygiene: Diagnosis and
management of shift maladaption syndrome. Presented at the 69th Annual
Meeting of the American Occupational Medical Association, Los Angeles,
California, May 1984.

(11.) Moore-Ede, M.C.
Applications of circadian rhythms to rotating shift work. Presented at
the 69th Annual Meeting of the American Occupational Medical Association,
Los Angeles, California, May 1984.

(12.) Borsook, D., Boulos, Z., Brennan, M., Porter, R., Moore-Ede, M.C.
The effect of sodium valproate on the free-running period in squirrel
monkeys. Presented at the First International Montreux Conference of
Chronopharmacology, Montreux, Switzerland, March 1984.

(13.) Frim, D.M., Boulos, Z., Moore-Ede, M.C.
Restricted but unsignaled daily food availability synchronizes anticipatory
behavior but fails to entrain other circadian rhythms in the squirrel monkey.
Presented at the Soc. for Neuroscience Annual Meeting, Oct. 1984, Anaheim CA.

(14.) Brennan, M.J.W., Moore-Ede, M.C., Borsook, D.
Regional circadian rhythms of benzodiazepine and GABA receptor binding in
rat brain. Presented at the 14th Annual Meeting of the Society for
Neuroscience, Anaheim, California, October 1984.

(15.) Brown, Emery N., Moore-Ede, M.C.
A method for assessing the compatibility of differential equation models
with observed circadian data. Presented at the Joint 25th Ann. Mtg. of the
Sleep Res. Soc. & the 10th Ann. Mtg. of the ASDC, Seattle WA, July 1985.

(16.) <u>Moline, M.L., Albers, H.E., and Moore-Ede, M.C.</u>
Absence of critical period for phenobarbital block of luteinizing hormone (LH) surges in anestrous short photoperiod hamsters. For presentation at the Annual Meeting of the Society for the Study of Reproduction, Ithaca, New York, July 1986.

(17.) <u>Moore-Ede, M.C., Klerman, E.B., Houpt, T.A.</u> Mathematical simulation of the effects of rotating shiftwork schedules on circadian sleep-wake cycles and alertness rhythms. To be presented at the Association of Professional Sleep Societies Annual Meeting, Columbus, O., June 1986.

(18.) <u>Houpt, T.A., Boulos, Z., Moore-Ede, M.C.</u> Circadian phase response curve to diazepam for hamsters in constant light. To be presented at the 16th Annual Meeting of the Society for Neuroscience, Washington, D.C., November 1986.

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