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AUTHOR(s)
James M. Krueger

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Department of Physiology and Biophysics
University of Tennessee, Memphis
894 Union Avenue
Memphis, Tennessee 38163

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Our previous research on sleep-promoting substances that accumulate in cerebrospinal fluid during wakefulness led to the discovery of the somnogenic activity of muramyl peptides (MPs). Our investigation of the mechanisms involved in MP-enhanced sleep led to the discovery of several other somnogenic substances over the past three years. Our findings indicate that it may be possible to develop new, more effective, and safer somnogenic agents using MPs and/or other substances involved in the regulation of normal sleep. Thus, our broad goal is to develop the information needed to ascertain if MPs or other endogenous sleep factors would be practical as somnogenic agents. In our preceding annual reports, we described results from 11 experiments; this year we report those from an additional five experiments, summarized as follows: 1) Murametide, a MP that induces interleukin-1 (IL1) production but also blocks IL1-induced fevers, potentiated IL1-induced fever and sleep when given at the same time as IL1. In contrast, when IL1

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19. ABSTRACT (continued)

was given 30 min after murametide, murametide completely blocked IL1-induced fevers but still potentiated IL1-induced sleep. 2) Previously, we proposed that MP induced sleep via a step involving IL1 production. IL1 also induces corticotropic-releasing factor (CRF) release, which leads to enhanced adrenocorticotropic hormone (ACTH) release and glucocorticoid release. Last year, we showed that α melanocyte-stimulating hormone (αMSH; i.e., $ACTH_{1-13}$) blocked IL1-induced sleep responses, and it was known that glucocorticoids turn off IL1 production. We therefore tested CRF in combination with IL1. CRF blocked IL1-enhanced sleep. 3) Several of the cytokines whose production are enhanced by MPs, IL1, interferon α_2, and tumor necrosis factor, are pyrogenic and somnogenic. IL6, another pyrogenic cytokine thought to be involved with many of the actions of IL1, was unable to alter sleep at doses that elicit fever. 4) We also showed that prolactin and two of its hypothalamic releasing factors, vasoactive intestinal peptide and peptide histidine-methionine, selectively enhanced rapid-eye-movement sleep. 5) Finally, we extended our observations of the changes in sleep that occur over the course of infectious disease. Last year, we provided the first study of sleep patterns during bacterial infection. This year, we studied several different species of bacteria and one fungal organism. All induced biphasic sleep responses. At first, sleep was enhanced; then, depending on the infectious agent 8-20 h later sleep was inhibited for periods up to 14 h. All of these results are discussed within the context of a sleep regulatory model that proposes how many of the sleep-promoting factors are linked to each other and how they may elicit their effects via various neuronal sets.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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Abbreviations

5HT	serotonin
aCSF	artificial cerebrospinal fluid
ACTH	adrenocorticotropic hormone
αMSH	alpha melanocyte-stimulating hormone
CCK	cholecystokinin
CFU	colony-forming units
CLIP	corticotropin-like intermediate lobe peptide
CNS	central nervous system
CRF	corticotropin-releasing hormone
CSF	cerebrospinal fluid
DSIP	delta sleep-inducing peptide
dsRNA	double-stranded ribonucleic acid
DWA	delta wave amplitude
EEG	electroencephalogram
GH	growth hormone
GRF	growth hormone-releasing factor
ICV	intracerebroventricular(ly)
IFN	interferon
IL1	interleukin-1
IL6	interleukin-6
IV	intravenous(ly)
LPS	endotoxin (lipopolysaccharide)
MDP	muramyl dipeptide
MP	muramyl peptide
NREMS	non-rapid-eye-movement sleep
PFS	pyrogen-free saline
PG	prostaglandin
PHI/PHM	peptide histidine isoleucine/methionine
POMC	proopiomelanocorticotropin
PRL	prolactin
REMS	rapid-eye-movement sleep
SC	subcutaneous
SF	sleep factor
SOM	somatostatin
SWS	slow-wave sleep
T_{br}	brain temperature
T_{co}	colonic temperature
TNF	tumor necrosis factor
VIP	vasoactive intestinal peptide
W	wakefulness

INTRODUCTION

The concept that sleep is regulated in part by humoral mechanisms is supported by experimental demonstrations of somnogenic substances in tissue fluids (reviewed, 19, 77, 84, 85, 88). These experiments began at the turn of the century with Legendre and Pieron (98) and Ishimori (62), who described the accumulation during prolonged wakefulness of substances in cerebrospinal fluid (CSF) that induce excess sleep when transferred to recipient animals. Although the chemical identities of these substances were never established, today over 30 putative sleep factors (SFs) have been identified (reviewed, 84). Most of these putative SFs are hormones, immunomodulators, and/or substances involved in endocrine and/or immune regulation.

Until recently, it was postulated that a SF, herein defined as any substances found within the body that alters sleep, would have biological actions specific to sleep and would act on central nervous system (CNS) executive sleep centers, which were also assumed to be concerned primarily with sleep regulation. However, a single CNS center necessary for sleep has not been demonstrated. Further, all SFs identified to date have multiple biological activities. Thus, a revision of the original assumptions is necessary. We have developed a model that links the sleep effects of many putative SFs in a sleep activational system (see general discussion).

Attempts to identify a sleep-promoting substance accumulating in CSF during sleep deprivation (Factor S) (129) resulted in the discovery of the somnogenic activity of muramyl peptides (MPs) (87). The sleep-promoting MP isolated from rabbit brain and from human urine has biochemical characteristics similar to those of Factor S obtained from CSF (86). Our investigations of the somnogenic actions of MPs have led us to describe several other substances

as somnogenic (reviewed, 84, 85, 88) over the past three years. These findings suggest to us that in the long run new, more effective and safer somnogenic agents can be developed using MPs and/or other substances involved in the cascade of events involved in normal sleep regulation. Therefore, the broad objective of our studies is to develop the information needed to ascertain if it is reasonable to propose MPs or other endogenous sleep factors as potential somnogenic agents. In the first year of our contract period (June 1, 1986-May 30, 1987), five sets of experiments were perforemd with this goal in mind; in the second year (June 1, 1987-May 30, 1988), six sets of experiments were performed. Results from those experiments are described in our annual reports dated June 1, 1987, and June 1, 1988. With the same objective in mind, this year we performed five additional sets of experiments. In this report, these experiments are numbered one through five as follows:

1) Our first experiment provides an example of a MP (murametide)-interleukin-1 (IL1) interaction. 2) In the second experiment, we show that corticotropin-releasing hormone (CRF) inhibits normal and IL1-enhanced sleep. 3) Next, we show that interleukin-6 (IL6), a pyrogenic cytokine, is not somnogenic. 4) In experiment four, we show that prolactin (PRL) and two of its hypothalamic releasing factors, vasoactive intestinal peptide (VIP) and peptide histidine-methionine (PHM) selectively enhance rapid-eye-movement sleep (REMS). 5) We extended our observations concerning sleep during infectious diseases and showed that several different microbes alter sleep.

METHODS

Sleep Assay

Male New Zealand White Pasteurella-free rabbits (approximately 3.5 kg) were surgically implanted with a lateral cerebral ventricular guide cannula, a thermistor, and electroencephalographic (EEG) electrodes. Stainless steel screws were placed over the frontal and parietal cortices. A 50,000-ohm calibrated thermistor (model 4108, Omega Engineering, Stamford, CT) was implanted over the parietal cortex to measure brain temperature (T_{br}). Insulated leads from the screws and the thermistor were routed to an Amphenol plug attached to the skull with dental acrylic (Duz-All, Coralite Dental Products, Skokie, IL). The guide cannula was placed in the left lateral ventricle, and its position was verified during implantation by monitoring pressure changes at the tip of an infusion needle. A minimum of 2 wk was allowed for recovery before the animals were habituated to the recording chambers.

The rabbits were housed in an animal facility on a 12:12 h light-dark cycle (lights on, 0600) at 21°C. Prior to experimentation, the animals were habituated to the recording chambers (Hotpack 352600, Philadelphia, PA) for at least two 24-h sessions. When experiments were scheduled, the rabbits were placed in the recording chambers the preceding evening, with the light-dark and temperature regimens of the recording chambers maintained under the same conditions as the animal facility. Food and water were available ad libitum.

Apparatus and recording. Each recording chamber contained an electronic swivel (Stoelting, Chicago, IL) suspended by a coiled shock-absorbing system. A flexible tether connected the swivel to the Amphenol connector on the rabbit's head, thus allowing freedom of movement. An accelerometer (Grass, SPA1,

Quincy, MA) attached to the shock-absorbing system provided an indication of body movement. The cables from the swivel and the accelerometer were connected to Grass 7D polygraphs in an adjacent room. These polygraphs recorded EEG, T_{br}, and body movement for each animal. The EEG for each rabbit was band-pass filtered with the 0.5-3.5 Hz (delta), 4.0-7.5 Hz (theta), 8.0-12.5 Hz (alpha), and 13.0-25.0 Hz (beta) frequency bands rectified and averaged for 1-min intervals by a Buxco model 24/32 data logger (Buxco Electronics, Sharon, CT). The averaged delta values served as a characterization of slow wave activity in non-rapid-eye-movement sleep (NREMS), a measure of sleep intensity. Additionally, amplitudes of EEG slow waves during 1-min epochs of NREMS for each of the first three postinjection hours were determined. The five samples of greatest magnitude in each hour were averaged for each rabbit during control and test periods. The ratio of theta to delta activity was also computed and displayed on the polygraph simultaneously with the EEG, T_{br}, and body movement to facilitate scoring of vigilance states. T_{br}s were also recorded using a data logger (Acrosystems 400, Beverly, MA), with values for each rabbit sampled at 10-min intervals. Colonic temperatures (T_{co}) were taken with a flexible thermistor (YSI, Inc., Yellow Springs, OH) at the time of injection and at the end of the 6-h recording session.

The EEG for each animal was visually scored for duration of each vigilance state (expressed as % time) for hourly intervals of the 6-h recording period. The following criteria were used: NREMS was associated with high-amplitude EEG slow waves, lack of body movement, low theta to delta ratios, and declining T_{br} upon entry. REMS was characterized by low EEG voltages, high theta to delta ratios, general lack of body movement with occasional twitches, and a rapid increase of T_{br} at onset. Wakefulness (W) was distinguished by

low voltage EEG, midlevel theta to delta ratios, frequent body movements, and a gradual increase in T_{br} after arousal.

Specific Experimental Protocols

1. Experiment No. 1: murametide-IL1 interactions

Test materials. Murametide (AcMur-L-ala-D-Gln-α methyl ester) was prepared by P. Lefrancier (96) and kindly provided by Institut Choay, Paris, France. Murametide was dissolved in pyrogen-free saline (PFS) (Abbott) for use. Two different lots of recombinant IL1β (rIL1β) expressed in Escherichia coli, purified to homogeneity, and provided by Biogen S. A. (Alan Shaw, lot #1) and Cistron Biotechnology, Inc. (Pine Brook, NJ, lot #2), were used. Each lot was tested for somnogenic and pyrogenic activity before use in the present experiments to determine biologically active doses. rIL1β was diluted with PFS, divided into individual aliquots, and frozen in polypropylene tubes until the day of its use. All needles, syringes, glassware, and solvents were sterile and nonpyrogenic.

Murametide (400-450 μg/kg) or rIL1β was diluted to the correct dose with PFS to give a 500-μl injection volume and was injected (ca. 30 sec) into a marginal ear vein. Two injections were given in each experiment. Murametide was always injected first; this was followed by injection of rIL1β after 0, 30, or 60 min. When either murametide or rIL1β was used alone, PFS was the other injectant. After injections, rabbits were returned to their cages, and recordings were obtained for the next 6 h. After the recording period, T_{co}s were again measured. At least 1 wk passed before a rabbit was used in another experiment. Control recordings from the same animals were taken on different

days under an identical protocol except that PFS was administered, providing a matched-pairs experimental design.

2. Experiment No. 2: CRF-IL1 interactions

CRF was purchased from Bachem, Inc. (Torrance, CA). IL1 (human recombinant IL1β; Cistron Biotechnology, Inc., Pine Brook, NJ) was a gift of Dr. C. Dinarello of Tufts University, School of Medicine. Both substances were dissolved in artificial cerebrospinal fluid (aCSF) containing 0.1% ascorbic acid immediately before injection.

Experimental protocol. Three groups of rabbits were used. All substances were injected into a lateral cerebral ventricle (intracerebroventricularly, ICV). Each injection was 12.0 μl in volume and took about 2 min. Each rabbit received two injections spaced 10 min apart in each experiment. The double injection protocol involved injections in combinations as follows: 1) Control recordings were made after two injections of aCSF for each group of rabbits. 2) Group 1 (n = 8) was injected with 0.1 μg CRF; Group 2 (n = 7) received 0.5 μg CRF, and Group 3 (n = 8) 1.25 μg CRF. These doses correspond to 0.02, 0.1, and 0.25 nmol CRF. Therefore, they were the same as, or similar to, the small doses of ovine CRF tested for EEG effects in rats by Ehlers et al. (44, 45). Ten minutes before each injection of CRF, animals received an aCSF injection. 3) All animals in each group also received on a separate day IL1 (20 ng) followed by aCSF. 4) When the effects of IL1 + CRF were tested, CRF was injected 10 min after IL1 in these combinations: 20 ng IL1 + 0.1 μg CRF (Group 1); 20 ng IL1 + 0.5 μg CRF (Group 2); and 20 ng IL1 + 1.25 μg CRF (Group 3). The 10-min interval between IL1 and CRF injections followed the procedure reported by Bernardini et al. (10) in an experiment studying the

effects of CRF on IL1-induced fever. The 6-h recording started after the second injection. The mean interval between two consecutive tests in a group was three days; the order of the four manipulations varied with the groups.

Behavioral observation. The effects of CRF on rabbit behavior were determined using a closed-circuit television system. Recording chambers were modified by replacing the door with a Plexiglas panel. Animals were placed in the modified recording chamber for a minimum of 30 min. After this acclimation period, the rabbits were injected with aCSF, 0.5 μg CRF (n = 4), or 1.25 μg CRF (n = 8). Their behavior was videotaped for 3 h postinjection. These tapes were subsequently scored visually at 1-min intervals, with behavior classified as sitting, lying, active (movement about the chamber, e.g., exploration), grooming, or ingesting (eating or drinking). In addition, any occurrence of rearing on hind legs was scored as a discrete event.

3. Experiment No. 3: IL6 is not somnogenic

IL6 (human rIL6 produced in Escherichia coli) was a gift of Genetics Institute (Cambridge, MA). IL1 (human rIL1β) was purchased from R & D Systems (Minneapolis, MN). Both substances were dissolved in aCSF (89) immediately before injection. Four doses of IL6 were tested: 20, 40, 80, and 200 ng (each delivered in 25 μl aCSF). Control recordings were made after injection of 25 μl aCSF on two different days. No statistical differences existed between the two control recordings; thus, they were averaged for statistical analysis. An additional control was tested; IL6 was heat-inactivated (70°C, 1 h) before injection. This treatment does not destroy contaminating endotoxin (> 4 < 8 EU/ml), thus allowing an assessment of whether responses are due to endotoxin.

4. Experiment No. 4: PRL, VIP, and PHI enhance REMS

Substances. ICV doses of VIP and PHM (Peninsula Lab, Inc.) were 0.01, 0.1 and 1.0 nmol/kg. The peptides were dissolved in aCSF (89) immediately before injection. The vehicle, i.e., aCSF, was injected ICV for control experiments in the same animals on different days. A volume of 25 μl was infused in 1 min. After the injections, the infusion needle was left in place for another minute, then the needle was replaced by a stylet.

PRL (ovine PRL, Sigma) was dissolved immediately before use in 0.16 M NaCl containing 0.03 M $NaHCO_3$ at pH 10.8 (adjusted with 8.0 M NaOH). The pH was lowered to 9.0 by the addition of 10 N HCl. The volume of subcutaneous (SC) injection (dorsal neck region) was 2.0 ml. Control recordings were carried out after SC injection of the vehicle.

Three doses of VIP were tested in the same group of rabbits (n = 13). The order of doses and the interval between tests (2-7 d) varied. Each day of VIP injections was preceded or followed by a day of baseline recording. The durations of vigilance states on the three baseline days were compared by a Friedman's test, and no significant differences were found. The baseline values were, therefore, averaged for each state of vigilance and for T_{br}, and the effects of VIP were compared to the mean control values.

The sleep effects of 0.01 and 0.1 nmol/kg PHM were studied in a different group of rabbits (n = 8). In this experiment, aCSF, 0.1 nmol PHM/kg, aCSF, and 0.01 nmol PHM/kg were injected on four consecutive days. The time spent in each state of vigilance on the two baseline days was compared by means of a Wilcoxon matched-pairs signed-ranks test, and no differences were found. The effects of these doses of PHM are, therefore, shown with respect to the mean baseline values. The 1 nmol/kg dose of PHM was tested on another group of

rabbits (n = 12); the baseline recording preceded the injection of PHM for these animals.

Two doses of PRL were SC injected into two groups of rabbits (45 IU/kg: n = 14; and 200 IU/kg: n = 8). Both groups of rabbits were recorded on three consecutive days; PRL was administered on day 2, and the vehicle was injected on days 1 and 3. The duration of vigilance states on days 1 and 3 were compared by means of the Wilcoxon matched-pairs signed-ranks test, and significant differences were not found. Therefore, the data for the vehicle days were averaged.

5. Experiment No. 5: effects of microbial challenge

For preparation of inocula of challenge organisms, S. pyogenes (ATCC 19615), E. coli (ATCC 25922), and C. albicans (Strain 310) were purchased as lyophilized cultures on Colti-loops (Scott Laboratories, Fiskeville, RI). Prewarmed blood agar plates were inoculated with S. pyogenes or E. coli and were incubated overnight at 37°C. S. pyogenes was incubated in a 5% CO_2 atmosphere to promote rapid growth of the organism. C. albicans was grown overnight on Sabouraud agar plates. Colonies were then transferred to sterile PFS to achieve a concentration of approximately 2×10^9 colony-forming units (CFU) per ml, initially estimated using a Klett-Summerson photoelectric colorimeter and later verified by plating serial dilutions of the microbial suspension on blood or Sabouraud agar plates. Separate groups of animals were inoculated with suspensions that were autoclaved prior to animal inoculation and confirmed to be free of live organisms by incubation on agar plates.

Rabbits were inoculated intravenously (IV) in the marginal ear vein with 0.1 to 0.9 ml of suspension containing approximately 1×10^8 CFU of S. pyo-

genes, 9×10^7 CFU of E. coli, or 4×10^7 CFU of C. albicans. Animals challenged with viable and heat-killed organisms were monitored for 48 h and 24 h postinoculation, respectively. Each rabbit was inoculated only once and was killed at the end of the experiment with IV T61 euthanasia solution (Hoechst-Roussel, Somerville, NJ).

Postmortem blood cultures were performed using samples obtained by cardiac puncture immediately after death. Blood was incubated for 24-48 h at 37°C in brain-heart infusion broth. An aliquot was then transferred to blood or Sabouraud agar plates for an additional 24 h incubation.

Statistical analysis

Data were analyzed with SPSS^X Information Analysis System. The amount of each state of vigilance across the 6-h recording period was compared to the baseline values by Friedman's test for k-related samples. Whenever the test indicated significant effects of the substances on any state of vigilance, the Wilcoxon matched-pairs signed-ranks test was used a posteriori to compare the baseline and experimental values on an hourly basis. The changes in the states of vigilance elicited by the various doses of a substance across the 6-h recording period were compared by Friedman's test to determine dose-effect relationships. The duration and frequency of REMS episodes on the experimental and baseline days were compared by the Wilcoxon matched-pairs signed-ranks test. Two-way ANOVA was used to determine if there were differences in the course of T_{br} between the baseline and experimental days. All tests were two-tailed, and the accepted level of significance was $p < 0.05$ for each test.

RESULTS

Experiment No. 1

Murametide administered alone failed to affect NREMS, REMS, EEG delta frequency amplitudes, or T_{br} (Table 1; Fig. 1). The rabbits continued to cycle normally through W, NREMS, and REMS episodes, and the normal changes in T_{br} that are coupled with changes of sleep states (81) were undisturbed. No abnormal behavior of the rabbits was observed after murametide treatment. In contrast, rIL1β administered without murametide pretreatment induced significant increases in NREMS, EEG delta wave amplitude, and T_{br} and decreases in duration of REMS (Fig. 2A, B, C), thereby confirming previous findings (143). However, the time course of rIL1β-induced fevers was dependent upon the particular lot of rIL1β used. Thus, after injection of lot #1, increases in T_{br} were of shorter duration than those observed after injection of lot #2 (Fig. 2; compare A and C to B), although the effects of both lots on sleep were similar.

The effects of murametide treatment on rIL1β-induced responses depended upon the time between murametide and rIL1β injections. If murametide was injected simultaneously with rIL1β, it significantly potentiated rIL1β-induced changes in T_{br} without greatly altering rIL1β-induced sleep responses (Table 1, Fig. 2A). In contrast, if murametide was administered 30 min before rIL1β was injected, IL1β-induced febrile responses were blocked, whereas rIL1β-induced NREMS, EEG delta wave amplitudes, and REMS responses were potentiated (Table 1, Fig. 2B). Placing 60 min between the murametide and rIL1β injections resulted in NREMS, EEG delta wave amplitudes, and temperature effects

similar to those observed after rIL1β alone, although REMS reduction was greater after the two injections compared to rIL1β alone (Table 1, Fig. 2C).

Experiment No. 2

Sleep. ICV administration of CRF increased W and reduced both NREMS and REMS (Fig. 3, Table 2). The changes in W and NREMS showed significant dose-effect relationships ($p < 0.05$ for NREMS and W, nonsignificant for REMS; Friedman's test across the effects of 3 doses). After injection of 0.1 μg CRF, durations of vigilance states were unchanged relative to controls. NREMS was virtually eliminated for 1 h and did not reach control values until post-injection hour 3 after injection of 0.5 and 1.25 μg CRF. Suppression of REMS in the first 2 h postinjection was followed by recovery to control values by postinjection hour 3 after 0.5 μg CRF. After injection of 1.25 μg CRF, REMS was abolished for 2 h and remained significantly lower than corresponding control values until postinjection hour 5. EEG slow wave amplitudes during NREMS were not greatly altered (Table 3).

As reported previously (125), NREMS increased and REMS decreased after ICV administration of 20 ng IL1 (Fig. 3, Table 2). W tended to decrease; however, changes in W were not significant at this dose of IL1 across the 6-h recording period in two out of the three groups studied. EEG slow wave amplitudes during NREMS increased compared to those recorded after injection of aCSF (Table 3). There were no significant differences in the sleep effects of IL1 among the three groups of IL1-treated rabbits (Friedman's test on changes in vigilance states and NREMS amplitudes).

ICV injection of CRF following central administration of 20 ng IL1 resulted in a dose-dependent inhibition of the IL1 effects on sleep ($p < 0.05$

for NREMS and W, nonsignificant for REMS; Friedman's test across the 3 doses). After administration of 20 ng IL1 + 0.1 μg CRF, NREMS and W were close to control values; thus, NREMS was significantly lower and W higher than after IL1 alone. REMS increased significantly across the 6-h recording period compared to values after IL1 injection, though it was still significantly lower than during control recordings. The effects of 0.5 μg CRF were similar to those of the low dose; i.e., the IL1-induced excess NREMS was abolished, W returned to control values, and the suppression of REMS by IL1 was significantly attenuated, though REMS remained less than normal (Table 2). The effects of 1.25 μg CRF in IL1-pretreated rabbits were such that no IL1-induced sleep changes were evident. NREMS was reduced to values indistinguishable from those after administration of this dose of CRF by itself. REMS was suppressed throughout the 6-h recording period. EEG slow wave amplitudes during NREMS were not significantly different when IL1 was followed by 0.1 μg CRF compared to IL1 alone. In contrast, 0.5 and 1.25 μg CRF significantly inhibited IL1-induced enhancement of EEG slow wave amplitudes during NREMS (Table 3).

Behavior. Preliminary tests in four rabbits failed to reveal any obvious behavioral response to 0.5 μg CRF. Therefore, only observations from eight rabbits injected with 1.25 μg CRF are reported below.

After administration of aCSF, the rabbits spent most of the time in a relaxed lying posture (Fig. 4). Time spent quietly sitting was negligible. Although all of the rabbits groomed, ate, and drank during the 3-h observation period, total time spent in these behaviors was minimal. The most frequent activity was exploration of the surroundings, and this activity included rearing on the hind legs. The occurrence of rearing decreased throughout the course of the observation period, along with trends for a reduction in activity and increases in time spent lying.

ICV injection of CRF abolished ingestion in all rabbits throughout the 3-h period. The time spent in a sitting posture increased significantly in postinjection hours 1 and 2 and returned to baseline level in postinjection hour 3. The increased time in sittng resulted from slight variations of the time in other behavioral categories. Although the fraction of time in lying posture did not change, qualitatively it appeared as if the rabbits were aroused since the ears were held erect instead of flattened against the head, as is the normal position. Grooming or exploring the environment also did not change after CRF injections, though the occurrence of rearing was greatly suppressed for 2 h and started to return only in postinjection hour 3.

Brain and body temperature. ICV injection of CRF elicited dose-dependent increases in T_{br} (Fig. 5, Friedman's test across the 3 doses). The 0.1 μg dose had no obvious effects, though Friedman's test indicated small but significant elevations of T_{br} at various times after CRF compared to aCSF. When 0.5 μg CRF was administered, the T_{br} values were slightly (0.1-0.3°C) and consistently above values during control recordings, resulting in a significant increase across the 6-h recording period. A persistent and significant T_{br} increase of about 0.5°C was observed in response to 1.25 μg CRF. T_{co}s taken at the end of the 6-h recording period were 40°C ($\pm$ 0.1) in response to 1.25 μg CRF, indicating definite hyperthermia (data not shown).

ICV administration of IL1 elicited fever responses characteristic of endogenous pyrogen (Fig. 5). T_{br} rose rapidly, producing a peak 1 h after injection. A second fever peak was observed in postinjection hour 3. T_{br} declined slowly after postinjection hour 3. However, it was still higher by about 1°C than the control values at the end of the recording period. The febrile response to 20 ng IL1 in Group 2 was significantly higher than in the other two groups (Friedman's test across the three groups). T_{co}s 6 h after

IL1 injection were significantly elevated (40.1-40.5°C; $p < 0.05$, Wilcoxon test) relative to control values in all three groups.

The effects of CRF on IL1-induced fever were dose-dependent (Fig. 5, Friedman's test across the 3 doses). Attenuation of fever began in postinjection hour 3 after the 0.1 μg dose of CRF. Thereafter, T_{br} remained slightly (0.3-0.4°C) below values recorded after IL1 injection. This reduction in fever was significant across the 6-h recording period. The attenuation of fever was apparent by postinjection hour 2 when 0.5 μg CRF was injected into IL1-pretreated rabbits. T_{br} declined for the remainder of the recording period, finally reaching values similar to those recorded after injection of this dose of CRF alone. This reduction of fever was significant across the 6-h recording period (Friedman's test). In contrast, 1.25 μg CRF failed to affect T_{br} responses to IL1 (Fig. 5).

Experiment No. 3

ICV injection of IL6 resulted in a dose-related febrile response (Fig. 6A, Table 4). T_{br} after the 20- and 40-ng doses remained essentially unchanged relative to values recorded after ICV aCSF. After the 80- and 200-ng doses, however, T_{br} increased significantly across the 6-h recording period (Friedman's test, $p < 0.05$ for each dose). The time courses of T_{br}s after the 80- and 200-ng doses differed, however. After 80 ng IL6, fever peaked by 2 h postinjection and slowly declined for the duration of the recording period (Fig. 6A). The 200-ng dose resulted in a fever only slightly higher than that evoked by the 80-ng dose (0.7°C *vs.* 0.6°C, respectively), but this fever was sustained for the entire 6-h recording period. ICV injection of heat-treated IL6 (200 ng) had no effect on T_{br} (data not shown). T_{co}s at the end of

the recording period were not different from control values after the 20-, 40-, and 80-ng doses (Table 4). After the 200 ng dose, however, T_{co} at the end of the 6-h recording period was increased relative to the beginning of the recording period (T_1 in Table 4) and was also significantly greater than T_{co} at the end of aCSF recording periods (Kruskal-Wallis test).

After ICV injection of 20-, 40-, and 80-ng doses of IL6, duration of NREMS for the 6-h recording period was at or below those obtained after aCSF injection (Fig. 6B, Table 4). The tendency across these doses was for reduced NREMS, though departures from control values never reached significance. NREMS after 200 ng IL6 was unchanged from controls across the 6-h recording period. After injection of heat-treated IL6, NREMS was reduced across the 6-h recording period relative to aCSF and the 200-ng dose (Friedman's test, $p < 0.05$). This significance resulted from the slight, but consistent, reduction in all rabbits, and the biological relevance of this reduction is questionable since the absolute value is virtually identical to those for 20-, 40-, and 200-ng doses (Table 4). Time spent in REMS was not different after injection of any dose of IL6 or heat-treated IL6 (Fig. 6C, Table 4).

Experiment No. 4

The baseline percentages of vigilance states showed little variation either between groups (Table 5) or across the 6-h recording period in any group (Figs. 7-9). NREMS occupied slightly more than half the recording time. The total duration of REMS was only a small fraction of the recording period, about 7%, i.e., 24-25 minutes in 6 h, which is characteristic of rabbits (81).

Effects of VIP. In response to ICV injections of various doses of VIP, the major changes in sleep-wake activity consisted of increases in REMS (Fig.

7, Table 5). REMS increased and W decreased significantly across the 6-h recording period after the 0.01 nmol VIP/kg. However, these changes within individual hours were small, with a significant effect on REMS only in post-injection hour 3. The largest increases in REMS were observed after 0.1 nmol VIP/kg. The rabbits spent about 38 min in REMS, a significant increase across the 6-h recording period. Significant increases in REMS were found in several postinjection hours, the first occurring in postinjection hour 2. W and NREMS were not significantly affected. ICV injection of the 1.0 nmol/kg dose of VIP elicited an increase in W for 1 h at the expense of both NREMS and REMS. Thereafter, REMS tended to increase. Although the changes were slight between baseline and experimental values within the individual hours, the increases in REMS were significant across the 6-h recording period. The frequency of REMS epochs increased significantly after each dose of VIP (Table 5).

Comparisons of the changes in sleep-wake states elicited by the three doses of VIP did not reveal a dose-effect relationship.

Effects of PHM. The changes in sleep-wake activity after PHM injection were similar to those elicited by VIP (Fig. 8, Table 5). The 0.01 nmol/kg dose promoted REMS and reduced W across the 6-h recording period, though changes between baseline and experimental values within individual hours were slight. Increases in REMS were also significant for the 6-h recording period after the 0.1 nmol/kg dose of PHM, while W decreased, and NREMS was not affected. The effects on REMS were significant in postinjection hours 2 and 4. The 1.0 nmol PHM/kg induced significant increases in W and decreases in NREMS for 1 h. REMS, however, was not suppressed. After postinjection hour 1, REMS increased, and this effect was significant across the 6-h recording period and for several individual postinjection hours. The frequency of REMS episodes during 6 h was significantly higher after each dose of PHM (Table 5).

The three doses of PHM did not show a significant dose-effect relationship.

Effects of PRL. SC injection of PRL also promoted REMS (Table 5. Fig. 9). There were significant increases in REMS and decreases in W across the 6-h recording period in response to administration of 45 IU PRL/kg. The changes in REMS were significant in postinjection hour 3. The 200 IU/kg dose of PRL induced substantial and prolonged increases in REMS and suppressions in W. The time spent in REMS was doubled, rising to about 50 min in 6 h. REMS increased significantly in postinjection hour 3 and thereafter remained elevated.

The effects of 200 IU PRL/kg were substantially larger than those of 45 IU/kg (Friedman's tests across 6 h; $p < 0.01$ for REMS, $p < 0.02$ for W, and nonsignificant for NREMS).

The 45 IU/kg dose increased the frequency of REMS episodes. Both frequency and duration of REMS episodes increased after 200 IU/kg (Table 5).

Effects of VIP, PHM and PRL on T_{br}. Statistical analyses (ANOVA) indicated some effects on T_{br} for 0.01 nmol VIP/kg ($F[1,23] = 6.02$ group effect) and 0.1 nmol VIP/kg ($F[1,23] = 14.4$ group effect), 1.0 nmol PHM/kg ($F[1,23] = 6.7$ group effect) and 200 IU PRL/kg ($F[1,23] = 6.99$ group effect). A tendency for increased T_{br} could be noted 3-4 h after the administration of both doses of PRL (time effect: $F[11,23] = 2.3$ for 45 IU PRL/kg and $F[11,23] = 2.27$ for 200 IU PRL/kg) (Fig. 9). The changes, however, were slight (maximum 0.3°C after 45 IU/kg and 0.4-0.5°C after 200 IU PRL/kg). In the experiments with VIP and PHM, the changes in T_{br} did not exceed 0.2-0.3°C (Figs. 7 and 8). It is doubtful that there is any biological significance to these minor changes in T_{br}.

Experiment No. 5

Intravenous administration of S. pyogenes, E. coli, or C. albicans to rabbits induced significant changes in sleep patterns. Rabbits inoculated with S. pyogenes exhibited a marked increase in the amount of NREMS during hours 4-24 postinoculation, although they continued to demonstrate a normal circadian pattern of decreased sleep during the nocturnal period (Fig. 10). Delta wave amplitudes (DWA) also increased from hours 4-8 postinoculation, but were significantly lower than control values during hours 26-44 postinoculation (Fig. 11). Inoculation of rabbits with the same dose of heat-killed organisms did not significantly alter NREMS or DWA (Figs. 10, 11).

In contrast to the prolonged sleep enhancement produced by S. pyogenes, inoculation of rabbits with E. coli increased the time spent in NREMS (Fig. 10) and DWA (Fig. 11) only during the first 2-4 h postinoculation. DWA then decreased below baseline levels during hours 6-32 after challenge. Moreover, the administration of the same dose of heat-killed E. coli produced effects that were essentially identical to those of the viable organism (Figs. 10, 11).

Rabbits inoculated with C. albicans demonstrated alterations in sleep that were qualitatively and quantitatively similar to those produced after challenge with S. pyogenes (Figs. 10, 11). As with S. pyogenes, administration of the same dose of heat-killed C. albicans did not significantly alter sleep (Figs. 10, 11).

In addition to the effects on NREMS, these agents also altered REMS in rabbits. REMS was attenuated during hours 8-28 after S. pyogenes administration, hours 2-10 after E. coli administration, and hours 6-34 after C. albicans administration (Fig. 12).

Seven of eight rabbits inoculated with S. pyogenes and seven of ten rabbits inoculated with C. albicans contained microbial organisms in postmortem blood samples collected 48 h after inoculation. In contrast, a positive blood culture was obtained from only one of seven rabbits sacrificed 48 h after inoculation with E. coli. However, for humane reasons, five of the rabbits challenged with E. coli were sacrificed prior to this time because of the severity of their clinical conditions; microbial organisms were recovered from the blood of four of these animals.

DISCUSSION OF SPECIFIC EXPERIMENTS

Experiment No. 1

Results presented here confirm previous findings to the extent that: 1) muramet ide itself is relatively nonpyrogenic (90, 130) and nonsomnogenic (90); 2) muramet ide has the capacity to block IL1-induced fevers (130); and 3) IL1-induced fever can be separated, in part, from IL1-induced sleep responses (89). New results presented here are that: 1) muramet ide does not block rIL1β-induced sleep responses; indeed, it potentiates sleep responses; and 2) when muramet ide and rIL1β are injected simultaneously, muramet ide potentiates the pyrogenic actions of rIL1β. It is likely that the interaction(s) between muramet ide and IL1 producing these effects are outside of the CNS since it was previously shown that central adminstration of muramet ide does not block the pyrogenic action of systemically administered IL1 (130). There are several possible explanations for these interactions, all of which are, by necessity, speculative.

Although there is no direct evidence at this time, it is possible that MPs bind to IL1 since a monoclonal anti-muramyl dipeptide (MDP) (NAM-L-Ala-D-iGln) antibody recognizes both MDP and IL1-like activity (23). Thus, within the context of current results, an IL1-murametide complex might be somnogenic but not pyrogenic, whereas IL1 bound to other MPs may be both pyrogenic and somnogenic.

It is possible that murametide differentially alters the transport of IL1 across the blood-brain barrier, perhaps preventing the movement of IL1 to sites responsible for stimulating fever while allowing IL1 to reach sites involved with sleep regulation. Similarly, murametide could block the transport/production of certain second messengers elicited by IL1, thereby inhibiting the stimulation of fever while permitting those messengers involved in sleep responses to have effect. Finally, perhaps murametide differentially alters IL1-induced changes in vascular permeability (33).

Another possibility is that murametide induces the production of short-lived endogenous antipyretics capable of blocking the pyrogenic action of IL1 but not its somnogenic activity. Indirect evidence supporting this possibility is that in human plasma, IL1-like activity peaks at the onset of NREMS when body temperatures are normally falling (113). Such an antipyretic substance is unlikely to be produced in the brain in response to murametide, since murametide neither lowers body temperature nor blocks IL1-induced fever when given centrally. Futhermore, certain endogenous antipyretics, e.g., α melanocyte-stimulating hormone (αMSH) (126) and CRF (127) block both IL1-induced fevers and sleep responses.

Previously, both the pyrogenic and somnogenic actions of certain MPs, e.g., MDP, have been discussed within the framework of a simple model postulating that MPs elicit enhanced IL1 production and release, thereby altering

prostanglandin (PG) metabolism, leading to sleep and fever responses. This model, although conceptually useful in a historical framework, is clearly insufficient for several reasons: 1) certain MPs, e.g., murametide and MDP, affect IL1 and PG production but have different effects on sleep and fever. 2) The role, if any, of PGs in fever regulation remains in debate (14), and certain PGs, e.g., PGD_2, enhance NREMS but induce hyperthermia in some species (80) and hypothermia in others (61, 151, 152), whereas other PGs, e.g., PGE_2, inhibit sleep (57). Thus, any model invoking PGs in either sleep or thermal regulation must specify the species of animal and the PG. 3) Several cytokines, including IL1α, IL1β, interferon 2α (IFNα), interferon γ (IFNγ), tumor necrosis factor α (TNF), and IL6, are all postulated to be endogenous pyrogens. Furthermore, there is evidence suggesting that some MPs differentially elicit various activities of IL1 (28). Perhaps murametide could preferentially elicit in vivo lymphocyte-activating factor activity above endogenous pyrogen activity. 4) Recently, the presence of IL1β mRNA, but not IL1α mRNA (48), and hypothalamic neurons containing IL1β-like immunoactivity (20) was demonstrated in the normal brain. These observations, coupled with current results, clearly indicate that the somnogenic and pyrogenic actions of MPs and cytokines are indeed complex. Nevertheless, it is clear from results presented here that the animal can elaborate specific responses to combinations of a MP and IL1, although the exact molecular and regulatory mechanisms involved remain to be determined.

Experiment No. 2

The major effect of CRF on sleep reported here is its ability to reduce both NREMS and REMS. These results are consistent with those of Ehlers et

al., who report that ICV administration of CRF induces dose-dependent EEG arousal in rats (44). In addition, CRF also has the capacity to reduce IL1-induced excess NREMS and fever and IL1-enhanced EEG DWA during NREMS. Although both CRF (0.5 and 1.25 μg) and IL1 decreased REMS when injected alone, the combination of the two lower doses of CRF (0.1 and 0.5 μg) with IL1 significantly attenuated the REMS suppressive effect of IL1. The partial restoration of IL1-suppressed REMS by CRF may be nonspecific. Thus, it has been suggested that suppression of REMS after IL1 is at least in part regulatory; the intense functioning of NREMS mechanisms inhibits the occurrence of REMS (88). IL1-induced fevers may also disturb REMS. CRF therefore may promote restoration of REMS by reducing NREMS pressure and fever. The 1.25 μg dose of CRF itself had a strong REMS suppressive action, and this dose also failed to reduce fever. After this dose, attenuation of IL1 effects on REMS could not be observed.

Bernardini et al. (10) report that CRF blocks the pyrogenic response to IL1, though CRF itself does not affect body temperature in normal rabbits. In contrast, the results obtained by Rothwell (136) suggest that CRF is involved in the development of IL1-induced fever in rats. Although only 0.1 and 0.5 μg doses of CRF reduced fever, and this effect appeared after a long latency and was much less pronounced in our experiments than expected on the basis of the report by Bernardini et al. (10), the antipyretic action could clearly be observed. Nevertheless, CRF itself tended to increase body temperature. This CRF-induced hyperthermia was obvious in response to 1.25 μg CRF. This effect may explain the failure of the large dose of CRF to attenuate IL1-induced fever. It is noted, however, that in spite of the significant hyperthermic action of 1.25 μg CRF, the febrile response to IL1 did not increase when administration of IL1 was combined with this dose of CRF. It seems, therefore,

that CRF has two independent thermoregulatory actions. On the one hand, CRF promotes thermogenesis (97) and thereby may increase body temperature. It is possible that this mechanism is involved in the development of fever in response to IL1, as suggested by Rothwell (136). On the other hand, CRF also has an antipyretic action that may promote the recovery of normal body temperature after the chill phase of fever is over. Data reported here are consistent with this hypothesis in the sense that the antipyretic actions of CRF (0.1 and 0.5 μg) were not observed until 2 h after IL1 + CRF injection.

CRF, at least in the doses tested in our experiments, did not produce dramatic changes in rabbit behavior. Ingestion was abolished, and this finding corroborates those found in rats (114) and sheep (137). The occurrence of rearing decreased after CRF treatment, the time spent in sitting posture increased, and the rabbits generally appeared to be more attentive than after injection of aCSF. Nevertheless, the proportion of time the animals were active or lying did not change after CRF. The increases in W therefore cannot be attributed to some behavioral action of CRF. In contrast to our results, others have previously reported that ICV injection of CRF induces pronounced behavioral changes, e.g., behavioral activation (45, 145). However, these effects are species- (43, 72) and dose-dependent (145, 155) and, furthermore, depend upon whether recipient animals are in familiar environments (78, 145) and free-moving or restrained (72). In addition to the well-known action of CRF on the adrenocorticotropic hormone (ACTH)-glucocorticoid pituitary-adrenal axis, CRF also elicits sympathetic activation (92), resulting in increased blood epinephrine and norepinephrine levels (21), elevated blood pressure and heart rate (58), enhanced thermogenesis (97), etc. The autonomic effects could contribute to the arousing action of the peptide.

Systemic administration of CRF fails to elicit the behavioral and autonomic responses observed after ICV injection (10, 92, 145, 155). The effects of ICV CRF also persist after hypophysectomy (21, 49, 78, 92, 114). These observations lead to the conclusion that the behavioral and autonomic effects of ICV CRF are mediated by CNS mechanisms independent of the ACTH-glucocorticoid axis. CRF-like immunoreactivity (109) and CRF binding sites (110) have been demonstrated in various regions of the CNS, suggesting a neurotransmitter or neuromodulator role for CRF. Excitation of hippocampal cells in slice preparations (3) and activation of locus coeruleus neurons in vivo (153) have been reported in response to CRF. Although these effects might have an important role in the behavioral and autonomic actions of CRF, the possible involvement of CNS opiomelanocortins, especially ACTH and αMSH, is considered. The similar distribution of CRF-like immunoreactivity, CRF binding sites, and opiomelanocortins in the brain suggests that these systems might be functionally related not only in the pituitary but also in the CNS (110). Release of ACTH from central opiomelanocortinergic neurons has been suggested (24), and CRF stimulates endorphin release from the hypothalamus in vitro (117). Although differences between the behavioral effects of centrally administered CRF and ACTH/αMSH exist, CNS opiomelanocortins may mediate at least some of the behavioral responses to CRF (43, 155). For example, microinfusion of anti-β-endorphin or anti-ACTH antisera into the arcuate nucleus and mesencephalic central grey results in inhibition of CRF-induced suppression of lordosis (144). The arcuate nucleus is also the origin of neurons that exhibit αMSH-like immunoreactivity and project to the septum. Various observations indicate that αMSH released in the septum in response to IL1-induced fever has a significant antipyretic effect and acts as a negative feedback for IL1 (100). The fever-related release of αMSH in the septum is paralleled by a

decrease in CRF concentration in the paraventricular nucleus (100). It is possible, therefore, that IL1 stimulates central αMSH release through CRF.

Comparisons between the effects of CRF and αMSH tested in experiments identical with the present study (126), in fact, indicate many similarities. Like CRF, αMSH also elicits dose-dependent suppression of sleep without increasing motor activity, inhibits IL1-induced enhancement of NREMS, attenuates IL1-suppression in REMS (in low doses), and decreases fever (126). Important differences, however, are also noted. αMSH inhibits IL1-induced NREMS less effectively than CRF. In contrast, the antipyretic action of αMSH is prompt and more pronounced than that of CRF, and αMSH also induces dose-dependent hypothermia in normal rabbits. Finally, αMSH increases the occurrence of stretching and yawning, and at higher doses sexual excitation occurs. The behavioral and autonomic effects of αMSH are postulated to be mediated by widely different brain areas (12). Although these responses appear together after ICV administration of αMSH, they are functionally independent and do not occur simultaneously when the endogenous αMSH/ACTH pathways are selectively stimulated. Therefore, the differences between the effects of αMSH and CRF do not exclude the possibility that central opiomelanocortinergic mechanisms are involved in the mediation of certain CRF effects.

It is also important to emphasize that all the central effects of CRF cannot be attributed to stimulation of central opiomelanocortin release. For example, it is likely that the activation of the sympathetic system resulting in increased metabolism is not related to opiomelanocortins (97). Activation of independent mechanisms may explain the thermoregulatory effects of CRF. Through the sympathetic system, CRF release might act as an effector mechanism in fever (136). Simultaneous stimulation of opiomelanocortinergic mechanisms may control the amplitude of the temperature rise and may be involved in de-

fervescence. Release of glucocorticoids may contribute to the attenuation of fever (160). In contrast, both stimulations of opiomelanocortinergic systems and sympathetic mechanisms may contribute to the arousing action of CRF. Although the mechanism for CRF effects remains to be determined, current results clearly indicate that CRF has the capacity to inhibit IL1-induced NREMS and to attenuate IL1-induced fever. We conclude that previously reported IL1-induced CRF release may be part of a negative feedback system that attenuates not only peripheral IL1 actions but also IL1 central effects.

Experiment No. 3

IL6 dose-dependently increased T_{br} and T_{co} with no effect on rabbit sleep-wake activity. Helle et al. report a rapid icrease in body temperature after IV injection of IL6 (58). The febrile response peaked 1 h after injection for both doses of IL6 they tested (0.5 and 5.0 μg/kg), and temperatures returned to control values by 3 h postinjection. In contrast, ICV injections resulted in a gradual increase in T_{br} for 2-3 h and a fever that was sustained for a longer time period. The differences in the time course of T_{br} between the present study and that of Helle et al. (58) confirm previously reported findings that febrile responses to pyrogens vary with route of administration. Shoham et al. found that fever induced by intravenous injection of IL1 was initiated sooner and had a shorter duration than fever after ICV administration of IL1 (143).

The CNS effects of cytokines vary. Unit activity of hypothalamic neurons is different in response to IL1β, TNFα, and IFNα_2 (142). Both TNFα (143) and IFNα_2 (82) elicit fever and excess sleep without increasing serum copper levels, whereas IL1β also induces hypercupremia in addition to febrile and somno-

genic responses (142). IL6 is the first cytokine reported to possess pyrogenic properties without somnogenic actions. These observations indicate that the central actions of individual cytokines are specific. Other lines of evidence also suggest a dissociation of pyrogenic and somnogenic effects elicited by immune response modifiers. For example, antipyretics inhibit IL1-induced fever without attenuating the enhancement of sleep (89).

Although ICV injected IL6 failed to promote sleep in our experiments, it remains possible that IL6 injected systemically or in high ICV doses has somnogenic effects. Nevertheless, the somnogenic actions of IL1 are clearly not mediated through IL6; IL1 strongly promotes sleep in doses that induce fever of similar magnitude to those reported here for IL6 (89).

Experiment No. 4

These results, in conjunction with other studies, indicate that promotion of REMS is the only consistent sleep effect of VIP in three species: rat (91, 123, 134), cat (39), and rabbit. PHM, which generally has biological effects similar to VIP and might act through VIP receptors (94), also mimicked the sleep effects of VIP in rabbits. The importance of endogenous VIP in the regulation of REMS is suggested by observations that ICV administration of anti-VIP antibodies selectively reduces REMS in rats (134). Further, incubation of CSF from sleep-deprived cats with anti-VIP antibodies abolishes the REMS-inducing properties of CSF samples (40). It seems, therefore, that a VIP-like substance with REMS-promoting activity accumulates in CSF during sleep deprivation. These findings suggest that VIP has a physiological role in REMS regulation.

VIP promotes NREMS in rats (91, 123, 134), an effect not demonstrated in either cats (39, 131) or rabbits (this study). Therefore, the effects of VIP and growth hormone-releasing factor (GRF) on sleep are similar in rats, whereas they differ in rabbits, since GRF elicits rapid increases of NREMS, followed by increases in REMS in both rats and rabbits (119). Previously, we suggested (119) that increases in NREMS after exogenously administered VIP might be a GRF-like action due to the relatively high cross-reactivity between these homologous peptides and receptors in rats (93). Exogenous VIP, in fact, has a GRF-like effect in rats, but not in healthy humans (75), as indicated by growth hormone secretory responses to VIP in vivo and in vitro (15, 103).

Besides promoting REMS, VIP and PHM also elicit increases in W. Increased motor activity in rats has been reported in response to ICV injection of large doses of VIP (63), suggesting that large doses of VIP also inhibit sleep in rats. Unlike promotion of REMS, W increased immediately after the administration of the peptides, and the effect vanished in 1 h. This might indicate direct action of VIP and PHM on some unknown sites that are not reached in effective concentration when small doses of the peptides are injected ICV. VIP binding sites and neurons containing VIP-like immunoreactivity have been described in various areas of the brain (see 135 for a review), and little is known of the functional significance of central VIPergic transmission. Nevertheless, it is worth mentioning that intrahippocampal injection of GRF induces EEG desynchronization and increases motor activity in rats (118). Although the hippocampus does not contain GRF-like immunoreactivity (140), it is rich in VIP binding sites and in neurons with VIP-like immunoreactivity (135). It is possible, therefore, that the hippocampus mediates the arousing effect of VIP and PHM, and the response to intrahippocampal GRF injection results from a cross-reaction between exogenous GRF and VIP-receptors.

The long and variable latency of REMS increases, the diffuse distribution of the effect over the 6-h recording period, and the lack of a clear dose-response relationship after administration of VIP or PHM suggest that these peptides do not act directly on REMS mechanisms. Serotonergic (131, 134) and cholinergic transmissions (40), both implicated in REMS regulation and known to interact with VIP, are probably not involved in the mediation of VIP effect on REMS. This prompted us to study whether PRL, the pituitary hormone released by ICV administration of VIP and PHM, increases REMS as observed after VIP and PHM. The PRL enhancement of REMS resulted from increased number and duration of episodes in response to 200 IU PRL/kg. VIP and PHM increased only the frequency of REMS episodes, corroborating the findings of Drucker-Colin et al. (40). The 45 IU/kg dose of PRL, however, only enhanced REMS frequency, not duration. It is likely, therefore, that the increased REMS episode duration is a function of the magnitude of the effect rather than an indicator of differences in the mechanism of action.

The latency of the REMS response to PRL was long; REMS increased in post-injection hour 3 after both doses of PRL. PRL is a normal constituent of CSF in man (5, 101, 116), rhesus monkey (73), rat (101), and rabbit (25). Fluctuations in PRL concentration in CSF correlate with a variation of PRL concentration in plasma (5, 73, 101, 116). PRL has a specific receptor-mediated transport mechanism in epithelial cells of the choroid plexus (158). Uptake of PRL by the choroid epithelial cells takes about an hour after systemic administration (157). Therefore, increases in PRL concentration in CSF follow rising plasma concentration with a time lag of about 1 h (73). Once in CSF, PRL is taken up by periventricular structures (95), and the PRL clearance rate from CSF is very slow (73). As a result, central effects of systemically administered PRL develop slowly. For example, changes in hypothalamic tyro-

sine hydroxylase activity occur 2.5 h after increased plasma PRL concentration (115). This time course is similar to REMS responses observed in our experiments. It is noted that the latency of increased REMS was shorter after the 0.1 nmol/kg dose of VIP and PHM than after PRL. This reduced latency appears to be at variance with the postulate that VIP and PHM act through PRL. However, PRL may reach the brain by routes other than transport through choroid epithelial cells. A significant amount of blood leaving the hypophysis is directed toward the hypothalamus (4), where pituitary secretions may be transported through tanycytes into the CSF, and/or pituitary hormones can leak through endothelial fenestrations of portal vessels into the subarachnoid space (8). It is likely that high concentrations of endogenous PRL released by ICV VIP or PHM into portal vessels reach the CSF or some hypothalamic action sites faster than PRL administered systemically.

The importance of the VIP/PHM-PRL system in physiological REMS regulation remains to be determined. As suggested by a multiple sleep-factor hypothesis (85), PRL might act in concert with other REMS-regulating substances. Relationships between episodic variations of plasma PRL concentrations and REMS-NREMS cyclicity were not found (154). However, the failure to observe a correlation between plasma PRL concentration and sleep states is expected if the kinetics of the PRL transport system are considered. That PRL and its hypothalamic releasing factors have the capacity of increasing REMS and that occurrences of major PRL secretion peaks are coupled to sleep (31, 140) indicate that PRL may be involved in sleep regulation.

Experiment No. 5

These experiments demonstrate that rabbits inoculated with microbial organisms exhibit marked time-dependent changes in sleep patterns that vary temporally depending on the particular organism that was administered. Treatment with the gram-positive bacterium S. pyogenes or the fungal organism C. albicans induced a prolonged stimulation of NREMS, as well as biphasic changes in DWA. Similar patterns of sleep also occurred after challenge with the gram-positive bacterium S. aureus (148). In contrast, the enhanced sleep induced by challenge with the gram-negative bacterium E. coli occurred with shorter latency and was of shorter duration than sleep changes occurring after challenge with fungal or gram-positive bacterial organisms. Furthermore, the somnogenic effects of S. pyogenes and C. albicans were prevented if similar doses of heat-killed organisms were administered, whereas the effects of E. coli were essentially the same regardless of whether viable or dead organisms were injected.

The three different types of infectious agents used in these experiments were compared because of differences in their biological structures. Fungal cell walls, for example, are composed largely of chitin, mannan, and other polysaccharides, whereas cross-linked MP chains are a major structural element in bacterial cell walls (54, 67). Gram-positive and gram-negative bacteria differ with regard to the amount and molecular arrangement of MPs in the cell wall and the presence or absence of endotoxin (lipopolysaccharide [LPS]) (54). Each of these microbial agents is also likely to elicit somewhat different immune responses from the infected host (36, 37, 42). Such considerations could be related to differences in the time course of sleep alterations after infectious challenge. Indeed, characteristic temporal patterns of fe-

ver, which in some cases have diagnostic value, accompany specific infectious conditions in humans (59, 106). Similarly, characteristic temporal pattern of sleep changes could accompany challenge with specific infectious agents. The rapid onset of the sleep changes induced by E. coli, for example, could be related to the presence of LPS in the gram-negative bacterial cell wall. Indeed, the IV admnistration of purified LPS or its lipid A moiety to rabbits elicits enhanced sleep within a latency of 1 h (83).

A potential relationship between infectious disease and sleep has previously been suggested on the basis of observations that bacterial cell wall components, such as MPs and LPS (68, 70, 81, 83, 87, 105), and endogenous immune response modifiers, including IL1, TNF, and IFNα_2 (82, 89, 132, 143, 146), are somnogenic. Furthermore, the production of these cytokines is altered during microbial infection (16, 22, 36, 46, 156). Thus, the physiological availability of both bacterial cell wall products and endogenous immune mediators is likely to be abnormally high during periods of microbial infection, and thus such substances could contribute to the production of enhanced sleep under these conditions. Indeed, differences in the time courses of the somnogenic effcts of microbial challenge could be related to endogenous levels of microbial products and/or to the immune response they elicit. For example, inoculation with S. pyogenes or C. albicans elicits somnogenic effects of long latency and duration, and infectious organisms can be recovered from the blood of most animals sampled 48 h after inoculation. This prolonged septicemia suggests the likelihood of microbial multiplication in vivo and, coupled with the ineffectiveness of inoculation with heat-killed organisms, could indicate that prolonged immune challenge supplied by the proliferating organisms could provide the stimuli that elicit sleep. Similar conclusions were suggested from studies in which rabbits received therapy with a bacteriocidal antibiotic

after IV challenge with viable S. aureus (148). Moreover, when high doses of heat-killed S. aureus (148) or purified S. aureus cell walls (65) are administered, sleep responses that are qualitatively similar to those elicited by viable inocula occur and are possibly mediated by the large antigenic challenge or the relatively high dose of MPs to which the animal is exposed.

In contrast to the effects of gram-positive and fungal agents, inoculation with either viable or heat-killed E. coli produced enhanced sleep of relatively short duration. Furthermore, few animals had positive blood cultures 48 h after inoculation, indicating that E. coli was rapidly cleared from the circulation of most rabbits. Because antibody against E. coli has been detected in the majority of rabbits sampled (79), an anamnestic immune response secondary to prior exposure could account for the rapid clearance of E. coli observed in this and other experiments (52). Thus, the stimulus for enhanced sleep could be relatively short-lived following E. coli administration. Similarly, the peak fevers produced in response to low IV doses of LPS abate within 3 h (35).

In addition to the initial phase of enhanced somnolence, biphasic DWA responses were also characteristic of the altered sleep patterns that occurred after infectious challenge. DWA responses similar to those described in these experiments have also been observed during recovery sleep subsequent to sleep deprivation (18, 129, 147). Although cell wall components of microbial agents and/or increased levels of immune response modifiers in the host (e.g., IL1) are likely to mediate the enhancement of NREMS and DWA that occurs after infectious challenge, the mechanisms responsible for DWA suppression are unclear. The only substance currently known to decrease DWA is αMSH (126). It is possible that microbial challenge is followed initially by the release of somnogenic cytokines and/or products of microbial degradation (66), and sec-

ondarily by the enhanced production of αMSH. αMSH could then act to reduce the intensity of sleep. Similarly, the magnitude of febrile responses may be regulated via the antipyretic actions of αMSH (6).

In addition to the effects on sleep, IV challenge of rabbits with S. pyogenes, E. coli, or C. albicans also produces several physiological alterations that are consistent with a state of infectious disease, including fever, neutrophilia, lymphopenia, and elevated plasma cortisol levels (150). Previous work has demonstrated that inoculation of rats with a live fungal organism induces temporally parallel increases in both body temperature and NREMS (76). However, in the rabbit, the temporal patterns of the febrile and hematologic effects were not closely correlated with the observed sleep changes. A temporal dissociation between the somnogenic, pyrogenic, and hematological effects of bacterial inoculation also occurred in rabbits challenged with S. aureus (148). In addition, previous reports indicate that antipyretic therapy blocked the febrile effects of MPs and IL1, but does not alter the somnogenic effects of these compounds (87, 89). These observations suggest that fever is neither an essential nor a sufficient mediator of the enhanced sleep associated with infectious conditions.

Our data thus demonstrate that complex time-dependent alterations in sleep occur subsequent to infectious challenge in rabbits and that these alterations vary depending on the challenge organism used. These descriptive observations suggest that altered sleep, like fever and acute phase changes, could represent a major clinical sign of infectious disease and provide the basis for future work aimed at elucidating the mechanisms responsible for altered somnolence during disease. Moreover, these findings imply that sleep could serve an adaptive function in combating infectious disease. It is possible, for example, that sleep, which is associated with a decreased metabolic

rate and muscular inactivity, would permit the animal to conserve metabolic energy, particularly during febrile conditions when overall energy requirements would be high (56). Currently, however, the survival value of enhanced sleep during disease states and our understanding of the precise mechanisms responsible for these effects remain topics for conjecture and future research.

GENERAL DISCUSSION

A Sleep Activational System

Many of the putative SFs alter the release and/or synthesis of other SFs; some of these interactions are illustrated in a proposed sleep activational system (Fig. 13). Although it is currently not possible to assign quantitative importance to each interaction or each SF, it is clear that such a system offers a wealth of feedback loops, providing both stability and many routes by which autonomic and behavioral influences could alter sleep. To illustrate how changes in this system may be elicited, we describe what may occur if an animal is pathologically challenged with gram-negative bacteria. If rabbits are injected IV with E. coli, NREMS will increase initially and then, after a few hours, decrease below control values; REMS is depressed throughout this period (149). Gram-negative bacteria contain LPS in their cell walls. This substance is likely to induce the sleep effects observed after gram-negative bacterial challenge because purified LPS, its lipid A moiety, and heat-killed gram-negative bacteria all elicit similar sleep responses (83, 149). Systemic injections of LPS also enhance CNS cytokine production (53). Several cytokines, e.g., IL1, enhance NREMS and inhibit REMS (89). Cytokines, in turn,

stimulate PG metabolism (33, 34). Some PGs, e.g., PGD_2, enhance sleep (61), while others, e.g., PGE_2 (57, 159), inhibit sleep; thus it is possible that cytokines alter sleep through their effects on PGs. However, cytokines also directly alter neuronal firing rates (142), and it remains unknown whether PGs are inter- or intracellular signals in regard to sleep. Nevertheless, it is likely that PGs are involved in cytokine-altered sleep. PGE_2, for example, inhibits IL1 production and induces IL1 receptor synthesis (2). thereby providing a somewhat complicated short-loop feedback system, possibly acting within the same cell. The timing of these events in vivo remains to be determined.

IL1 is also involved in another feedback loop involving many cells and endocrine glands. Thus, IL1 enhances the release of hypothalamic CRF in vivo (9, 13, 138). CRF in turn leads to altered pituitary ACTH release, and ACTH induces adrenal release of glucocorticoids. CRF, ACTH, αMSH, and glucocorticoids all inhibit sleep. Glucocorticoids also inhibit IL1 production and enhance IL1 receptor synthesis (2, 99). Bacterial infections (148) and LPS itself also enhance glucocorticoid release, possibly via the mechanism outlined above.

LPS increases the release of both insulin (50, 161) and growth hormone (GH) (55), as does central administration of IL1 (26, 30). Insulin, in turn, has the capacity to enhance NREMS (29). The IL1-stimulated release of pituitary GH (11) may be mediated via hypothalamic GRF. GRF also increases GH release, and GH itself enhances REMS. In large doses, GH inhibits NREMS (41, 108); it also inhibits GRF release. GRF, however, enhances both NREMS and REMS (45, 119). Finally, IL1 may inhibit the release of pituitary PRL (11), although this finding is not clearly substantiated (133). PRL enhances REMS (121).

If the normal physiology of the animal is pathologically disturbed, as in our example, by challenge with gram-negative bacteria, many of the substances in the sleep activational system are altered. Moreover, the overall influence of such perturbations may vary, depending on the initial state of the system at the time of challenge. Thus, one would anticipate that the somnogenic effects of LPS administered during peak circadian concentrations of glucocorticoids would differ from effects observed after administration when glucocorticoid concentrations are low. Similarly, if an animal is stressed, thereby activating the CRF/ACTH/glucocorticoid system, the effects of SFs operating via cytokines may be altered.

This sleep activational system has the advantage of providing stability to sleep/wake regulation due to the multiple feedback loops. Thus, normal daily variations in the challenges that an animal encounters will not greatly alter sleep. In fact, it is emphasized that all experimental conditions that reliably produce increases in sleep are not physiologic,[1] e.g., prolonged sleep deprivation (71), infectious disease (148), excessive exercise (post-marathon) (141), and prolonged starvation (32). The major initial site of action of each of these manipulations within the sleep activational system (Fig. 13) may differ. However, it is possible that the entire scheme, including the bacterial and viral products shown, is involved in each of these paradigms. Further, each of these paradigms has also been used in various "stress" experiments; a large literature indicates that stress alters immune function and susceptibility to disease (reviewed, 1). Many SFs, first de-

[1]One possible exception to this rule is the transient increase in sleep observed upon exposure to mild increases in ambient temperatures. However, this response may be viewed as one facet of behavioral thermoregulation, in that metabolic rate during sleep drops below basal metabolic rate; thus, the heat dissipation demands upon warming are reduced if the animal sleeps (124).

scribed as immune response modifiers, are now recognized as CNS products; e.g., IL1-like activity is in normal CSF (102), IL1-mRNA is constituently expressed in the brain (48), and IL1-immunoreactive cells are found in the hypothalamus (20). Moreover, bacteria (7) and substances of bacterial origin (64) translocate across the intestinal wall, and bacterial products are found in the brain (86, 162), suggesting a constant exposure to such substances. Each of the paradigms mentioned above is likely to amplify the degree of exposure and/or responsiveness to these substances. Sleep deprivation, for example, leads to a reduced intestinal crypt cell production rate (128); this in turn could influence the rate of exposure to intestinal flora and bacterial products. Sleep deprivation also leads to altered IL1 levels in plasma (112) and to altered GH release (111). Regardless of the impact of sleep deprivation or other challenges, this sleep activational system probably exhibits a basal degree of activity under normal conditions.

The sleep activational system presented (Fig. 13) is provisional and partial; many caveats and points of explanation follow. 1) The cellular sites of either SF production or action are not illustrated, primarily because these remain to be determined in reference to their effects on sleep. However, with the exceptions of LPS and dsRNA, all are known constituents/products of the brain. 2) Some putative SFs that may affect this system are not included, e.g., adenosine. Further, some interactions between the SFs are not included. PGs, for example, may be involved at multiple sites in this system as intracellular messengers. PGs appear to be involved in GRF release (74) and actions (47) and in the IL1-induced release of insulin (27). 3) The sleep activational system does not necessarily represent the primary regulatory pathways for each substance. Further, inputs to the system such as sleep deprivation, food intake, and infectious disease may simultaneously affect

several SFs, as illustrated in the example of gram-negative infection. 4) With the exception of serotonin (5HT), the roles of neurotransmitters are not illustrated; the reader is referred to a review by Koella (77). 5) Similarly, the interactions of these SFs with circadian rhythms are not shown; these have been treated theoretically elsewhere (17). 6) Some of the information concerning the interactions between SFs illustrated in Fig. 13 is derived from in vitro experiments in which tissue of non-CNS origin was used, e.g., PGE_2 inhibition of IL1 production. Whether these relationships occur in the brain remains to be determined. 7) Some of the SFs may directly affect sleep via mechanisms that do not involve the pathways shown. For example, MPs directly alter firing rates of neurons (38); such an effect need not involve altered IL1 and PG production. 8) Some of the SFs alter both NREMS and REMS, while others are more selective. Some of these differences are dose- and species-dependent. Thus, VIP in rats induces both NREMS and REMS, possibly because VIP interacts with GRF receptors in this species (103); however, in rabbits and cats, VIP only induces REMS (121). Similarly, certain MPs, e.g., MDP, elicit both REMS and NREMS at low doses; but as the dose is increased, REMS is inhibited and NREMS increases (69, 104).

Sleep Factors and Their Interactions with Neuronal Sets

The sleep activational system illustrated in Fig. 13 shows possible interactions between many SFs. However, it is well known that all of the putative SFs illustrated have other biological activities, and some of their activities are not normally observed during sleep. For example, IL1 and some PGs are pyrogenic, yet body temperatures decrease upon entry into NREMS (122, 159). Thus, we have constructed a second model (Fig. 14) to illustrate how

specific sleep responses may be elicited from multiple SFs having multiple biological activities.

Chemical or electrical stimulation of many brain structures elicits sleep or sleep-like EEG activity. Lesions in various brain regions induce hypersomnia or insomnia, followed by partial or complete recovery of sleep. Further, sleep involves changes in many behavioral, psychological, and physiological variables. These variables, in turn, may modulate brain areas implicated in sleep regulation. Such considerations led McGinty to conclude that sleep is regulated via numerous independent neuronal networks at all levels of the neuraxis (107). Our model (Fig. 14) depicts three such neuronal sets (A-C), each contributing to a propensity to sleep, operating in parallel and being interconnected at some level within the neuraxis. Any substance that acts on these neuronal sets to alter sleep/wake activity is considered a SF (W-Z, Fig. 14).

We envision that a single neuronal set will interact with more than one SF (2 in the case of Fig. 14) and that the degree to which a neuronal set is stimulated depends upon the concentrations of the SFs interacting with it. Similarly, a single SF may interact with more than one neuronal set, and the actions of a SF on one neuronal set may differ from its actions on another neuronal set. Thus, a SF may be excitatory in one neuronal set while inhibitory in another (e.g., IL1 generally depresses hypothalamic warm-sensitive neurons while exciting cold-sensitive neurons [142]). This type of effect on different neuronal sets may contribute to the inhibitory effects observed after injection of combinations of SFs reported by Inoué (60). SF affinities for the different neuronal sets may also vary. Such interactions are well known and, indeed, provide a pharmacological basis for receptor classification. It is, therefore, easy to envision why some SFs (e.g., delta sleep-in-

ducing peptide, desacetyl αMSH) have somnogenic activity only in a narrow dose range.

For the purpose of illustration, we have assigned hypothetical numerical values to the outputs of each neuronal set in terms of three different biological activities. These values represent relative contributions of individual neuronal sets to the variable shown. It is emphasized that if one chose different neuronal sets, the relevant biological variable would be different; e.g., another neuronal set may influence sleep and osmotic balance. It is assumed that if the combined value of the outputs from the three neuronal sets illustrated for an individual activity reaches 9, then an increase in that activity will occur[2]. The output from any individual neuronal set is variable and depends on the degree of SF stimulation. Thus, the impact of SFs on sleep can be described as follows.

1. Some neuronal sets contribute more (have a larger output value; e.g., Fig. 14, A, B > C) to sleep propensity than others; thus, some SFs will be more important than others in reference to sleep (e.g., SF-X has a greater potential to enhance sleep than does SF-Z, Fig. 14).

2. If all neuronal sets are operating at a minimal level, the total sleep propensity output is less than 9; thus, these sets of neurons will not induce sleep.

3. Although the output of two neuronal sets may remain constant, maximal stimulation of a third neuronal set can permit the sleep threshold to be reached; e.g., the exogenous admnistration of a SF may cause excess sleep.

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[2]This model is formally set up as a threshold system; i.e., a certain level of activation must be reached before effects are observed. This was done for ease of illustration. Similar models in which each neuronal set continuously provides some degree of activation/inhibition for each biological activity that it affects can also be constructed. The same conclusions are reached, although the quantitative description of this is more lengthy and is unnecessary for this discussion.

4. In contrast, if sets B and C are at minimal output levels, stimulation of set A alone by SF-W will not initiate sleep (although it would if sets B and C were operating at higher levels). For example, it has been suggested that the somnogenic effect of exogenous delta sleep-inducing peptide requires an already existing sleep pressure (120).

5. Total sleep propensity would be reduced if one set were lesioned. However, sleep would be possible if the remaining sets were driven to a greater degree. Thus, an inherent feature of this model is that no individual neuronal set causes sleep, but individual sets do contribute to overall sleep propensity. One could, therefore, envision that sleep recovery after a lesion could occur as a result of a gradual increase in SFs that drive the nonlesioned sets and/or altered receptor affinities for the remaining SF receptors.

Another concept depicted in Fig. 14 is that any given neuronal set is involved in physiological functions other than sleep. To illustrate how responses specific to sleep could result from such a construction, we have included two additional physiological parameters in Fig. 14, body temperature and blood glucose levels, and, as in the case of sleep propensity, assigned hypothetical numerical values for the outputs from each neuronal set for these functions. Again, we assume that a collective value of 9 (from all 3 sets) must be reached before a specific function is altered by these sets. We will consider two different scenarios. In the first case, let us assume that the concentrations of SFs W-Z are such that the output for A is 6, for B, 2, and for C, 1. In such a condition, the combined outputs of sets A-C for sleep would be 9, thus increasing sleep propensity. In contrast, the combined outputs for body temperature would be 6 (because neural set A cannot contribute more than 3 to this activity), and the combined output for blood glucose would be 5; thus, these latter two parameters would not be affected by this particu-

lar combination of SF concentrations; thereby a specific effect on sleep would be observed. In contrast, if we choose a scenario such that the concentrations of SFs permit an output of 3 for A, 1 for B, and 5 for C, then the summated outputs from the three sets for sleep would be 6, that for body temperature 9, and that for blood glucose 8; thus, only body temperature would be enhanced under this particular combination of SF concentrations. From these two examples, it is easy to construct conditions that would produce specific blood glucose responses.

It is likely that there are multiple sites in the brain hierarchically arranged to regulate sleep. At each level, a scheme similar to that of Fig. 14 could be developed. However, the complexity is likely to be different at each level of the hierarchy. At various levels, many of the inputs (e.g., SF concentration) into these neuronal sets might be dependent on past sleep/wake activity. No set would be specific for any function, such as sleep, and all would be involved in regulation of two or more physiological variables. On the other hand, from a theoretical point of view, consideration of the sleep propensity outputs of the neuronal sets illustrated in Fig. 14 is important, as it has bearing on whether it is reasonable to postulate the existence of a SF specific for sleep. If the sleep outputs from these neuronal sets either diverge or operate in parallel to produce sleep, there would be little chance of finding a SF with biological activity confined to sleep because the same model would apply to the subsequent parallel/divergent pathways. In contrast, if the outputs of individual components of the model converge to influence a single common pathway involved only in sleep regulation, then it would be possible that the summated output of sets A-C affects the concentration within that pathway of some substance which, if made available to the appropriate intracellular sites, could enhance sleep. However, to date, neither such a

substance nor such a specific sleep pathway has been identified. Further, we consider such a brain construction (single final common pathway for a vigilant state) unlikely for any complex behavior such as sleep in view of the effects of sleep on many physiological functions.

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TABLE 1: **Effects of murametide and combination of murametide and interleukin-1 on rabbit sleep, electroencephalographic amplitude, and brain temperature.**

Time between injections	n	% SWS[2] Control	% SWS[2] Experimental	% REMS[2] Control	% REMS[2] Experimental	EEG delta wave amplitudes[2] Control	EEG delta wave amplitudes[2] Experimental	T_{br} at hour 1 Exp.-Control
0 minutes[1]								
rIL1β	4	42.8 ± 3.3	55.4 ± 2.9*	7.6 ± 1.4	6.8 ± 1.2	113.7 ± 7.6	137.8 ± 5.5*	0.33 ± 0.1
Murametide + rIL1β	4	42.8 ± 3.3	57.5 ± 3.1^{+}	7.6 ± 1.4	4.5 ± 0.9	113.7 ± 7.6	143.7 ± 8.5^{+}	0.65 ± 0.2*
30 minutes[1]								
rIL1β	4	57.5 ± 3.3	71.7 ± 3.9*	2.9 ± 1.4	0.14 ± 0.1*	88.5 ± 8.5	115.1 ± 12.2	0.85 ± 0.2*
Murametide + rIL1β	8	50.6 ± 2.5	76.8 ± 3.9†	2.9 ± 0.5	0.04 ± 0.04†	83.3 ± 6.6	115.0 ± 13.8†	-0.14 ± 0.1
60 minutes[1]								
rIL1β	4	42.7 ± 3.1	54.7 ± 2.8*	7.7 ± 0.7	6.6 ± 0.8	89.2 ± 8.7	107.5 ± 10.7*	0.43 ± 0.2
Murametide + rIL1β	4	42.7 ± 3.1	53.6 ± 4.5*	7.7 ± 0.7	2.8 ± 0.8*	89.2 ± 8.7	107.0 ± 12.1*	0.55 ± 0.3*
Murametide alone		50.1 ± 3.5	51.1 ± 2.6	5.3 ± 1.1	5.1 ± 1.3	89.1 ± 6.1	90.3 ± 6.4	-0.29 ± 0.2

[1]Lot #1 rIL1β used for assays when 0 and 60 min separated murametide and rIL1β injections; lot #2 rIL1β used when 30 min separated murametide and rIL1β injections.

[2]Three-hour means (± SEM) for slow-wave sleep (SWS), rapid-eye-movement sleep (REMS), and EEG delta wave amplitudes for different doses and combinations of murametide and recombinant interleukin-1β (rIL1β).

*$p < 0.05$, $^{+}p < 0.01$, $^{\dagger}p < 0.001$, Friedman test.

Table 2. Effects of corticotropin-releasing factor (CRF), interleukin-1 (IL1), and IL1 + CRF on rabbit sleep-wake activity.

	Percent Time Spent in Vigilance States*		
	NREMS	REMS	W
Group 1 (n = 8)			
20 ng IL1 + 0.1 μg CRF	51.1 ± 1.9†	4.1 ± 0.5+†	44.8 ± 2.2†
20 ng IL1	60.2 ± 1.6+	2.7 ± 0.5+	37.1 ± 1.7
0.1 μg CRF	49.1 ± 1.3	6.7 ± 0.7	44.1 ± 1.7
aCSF¶	49.0 ± 1.4	8.8 ± 0.8	42.2 ± 1.6
Group 2 (n = 7)			
20 ng IL1 + 0.5 μg CRF	46.2 ± 1.9†	3.5 ± 0.6+†	50.3 ± 2.1†
20 ng IL1	61.5 ± 2.1+	1.7 ± 0.4+	36.9 ± 2.1+
0.5 μg CRF	36.3 ± 2.9+	6.9 ± 1.0+	56.7 ± 3.5+
aCSF	44.3 ± 1.5	10.2 ± 1.2	45.5 ± 2.4
Group 3 (n = 8)			
20 ng IL1 + 1.25 μg CRF	37.2 ± 2.8+†	1.6 ± 0.4+	61.2 ± 3.0+†
20 ng IL1	60.2 ± 1.6+	2.7 ± 0.5+	37.1 ± 1.6
1.25 μg CRF	38.2 ± 3.3+	2.9 ± 0.7+	59.2 ± 3.6+
aCSF	48.0 ± 1.5	8.1 ± 0.7	43.9 ± 2.0

*Values are means ± SE across 6-h recording periods; NREMS, non-rapid-eye-movement sleep; REMS, rapid-eye-movement sleep; W, wakefulness.

¶artificial cerebrospinal fluid.

+Friedman's test, $p < 0.05$ vs. aCSF.

†Friedman's test, $p < 0.05$ vs. IL1.

Table 3. Effects of corticotropin-releasing factor (CRF), interleukin-1 (IL1), and IL1 + CRF on amplitudes (μV) of EEG slow waves (0.5-3.5 Hz) during NREMS*.

	Hour 1	Hour 2	Hour 3
Group 1 (n = 8)			
20 ng IL1 + 0.1 μg CRF	246.9 ± 23.3	258.7 ± 21.2^{+}	251.7 ± 21.9
20 ng IL1	246.4 ± 21.5^{+}	268.0 ± 20.9^{+}	254.5 ± 20.4^{+}
0.1 μg CRF	216.2 ± 16.9	221.1 ± 17.5	224.6 ± 17.4
aCSF¶	213.1 ± 17.3	215.3 ± 16.6	218.6 ± 16.3
Group 2 (n = 7)			
20 ng IL1 + 0.5 μg CRF	208.7 ± 18.5$^{\dagger+}$	225.2 ± 21.3†	214.0 ± 18.9†
20 ng IL1	256.7 ± 19.5^{+}	276.4 ± 21.9^{+}	269.4 ± 20.1^{+}
0.5 μg CRF	216.7 ± 8.3	209.0 ± 16.1^{+}	196.5 ± 13.4
aCSF	214.8 ± 16.5	228.9 ± 17.9	218.5 ± 17.8
Group 3 (n = 8)			
20 ng IL1 + 1.25 μg CRF	181.5 ± 14.5$^{\dagger+}$	209.5 ± 19.2†	195.1 ± 17.1†
20 ng IL1	234.0 ± 21.0^{+}	253.6 ± 21.2	254.4 ± 20.4
1.25 μg CRF	176.6 ± 25.0	208.1 ± 23.1	221.1 ± 20.0
aCSF	213.1 ± 17.3	215.3 ± 16.6	218.6 ± 17.6

*Non-rapid-eye-movement sleep; values are means ± SE of five samples for each rabbit (see METHODS).

¶Artificial cerebrospinal fluid.

$^{+}$Wilcoxon test, $p < 0.05$ vs. aCSF.

†Wilcoxon test, $p < 0.05$ vs. IL1.

Table 4. Effects of interleukin-6 (IL6) and IL1β on vigilance states (non-rapid-eye-movement sleep [NREMS], rapid-eye-movement sleep [REMS], wakefulness [W]) and colonic temperature. Values are means ± SE of six rabbits across 6-h recording periods.

	PERCENT TIME SPENT IN VIGILANCE STATES			COLONIC TEMPERATURE*	
	NREMS	REMS	W	T_1	T_2
aCSF+	52.0 ± 1.7	6.8 ± 0.8	41.2 ± 2.2	39.1 ± 0.1	39.3 ± 0.1
20 ng IL6	46.9 ± 1.3	8.1 ± 0.8	45.7 ± 1.7	39.1 ± 0.1	39.3 ± 0.1
40 ng IL6	46.3 ± 1.4	8.6 ± 1.0	45.1 ± 2.0	39.0 ± 0.1	39.4 ± 0.2
80 ng IL6	43.4 ± 1.9	8.4 ± 0.9	48.2 ± 2.3	39.0 ± 0.1	39.4 ± 0.1
200 ng IL6	53.3 ± 1.6	7.1 ± 0.8	39.6 ± 2.0	39.2 ± 0.1	40.2 ± 0.1#
HEATED†	44.0 ± 1.7¶	8.3 ± 0.8	47.8 ± 2.1	39.2 ± 0.07	39.4 ± 0.1
5 ng IL1β	75.3 ± 2.6¶	2.2 ± 0.6¶	22.4 ± 2.1¶	39.1 ± 0.1	40.4 ± 0.3¶
aCSF**	54.0 ± 2.0	11.5 ± 5.0	34.5 ± 2.5	39.2 ± 0.05	39.4 ± 0.03

*T_1 = initial colonic temperature; T_2 = colonic temperature at end of 6-h recording period.

+aCSF = artificial cerebrospinal fluid.

†heat-denatured 200 ng dose IL6.

¶$p < 0.05$ vs. aCSF, Wilcoxon test.

#$p < 0.05$ vs. aCSF, Kruskal-Wallis test.

**aCSF = artificial cerebrospinal fluid control for IL1β.

TABLE 5. Percents (mean ± SE) of W, NREMS and REMS, mean (± SE) durations and frequencies of REMS episodes during the 6-h recording periods after ICV injection of aCSF, doses of VIP and PHM, and after SC administration of PRL or vehicle. Asterisks denote significant differences ($p < 0.05$) with respect to control values (Friedman tests across 6 h for the vigilance states; Wilcoxon test for the duration and frequency of REMS).

SUBSTANCE DOSE	N	W (%)	NREMS (%)	REMS (%)	REMS DURATION	REMS FREQUENCY
aCSF	13	42.5 ± 1.3	50.2 ± 1.2	7.2 ± 0.5	1.6 ± 0.1	15.1 ± 0.6
VIP 0.01 nmol/kg	13	39.1 ± 1.2*	51.7 ± 1.2	9.2 ± 0.6*	1.6 ± 0.1	18.8 ± 1.0*
VIP 0.1 nmol/kg	13	40.5 ± 1.5	48.8 ± 1.3	10.6 ± 0.7*	1.9 ± 0.2	21.0 ± 1.3*
VIP 1.0 nmol/kg	13	41.8 ± 0.9	49.5 ± 0.7	8.2 ± 0.4*	1.9 ± 0.2	16.3 ± 0.9*
aCSF	8	42.8 ± 2.2	50.4 ± 2.0	6.8 ± 0.6	1.6 ± 0.1	15.5 ± 1.4
PHM 0.01 nmol/kg	8	38.1 ± 2.4*	51.7 ± 1.7	10.2 ± 1.1*	1.7 ± 0.1	21.8 ± 2.3*
PHM 0.1 nmol/kg	8	37.9 ± 1.6*	52.2 ± 1.5	9.9 ± 0.7*	1.7 ± 0.1	22.8 ± 2.3
aCSF	12	39.5 ± 1.6	54.7 ± 1.6	5.7 ± 0.4	1.5 ± 0.1	12.9 ± 1.1
PHM 1.0 nmol/kg	12	37.0 ± 2.0	53.0 ± 1.9	10.0 ± 0.7*	1.6 ± 0.1	20.3 ± 1.5*
VEHICLE	14	41.0 ± 1.3	51.3 ± 1.3	7.8 ± 0.9	2.0 ± 0.2	12.5 ± 1.1
PRL 45 IU/kg	14	37.1 ± 1.8*	52.5 ± 1.5	10.4 ± 1.3*	2.2 ± 0.2	15.4 ± 1.7*
VEHICLE	8	42.7 ± 1.5	50.1 ± 1.3	7.2 ± 0.5	1.8 ± 0.1	12.9 ± 1.1
PRL 200 IU/kg	8	34.4 ± 1.6*	51.8 ± 1.5	13.9 ± 1.2*	2.5 ± 0.2*	21.3 ± 1.5*

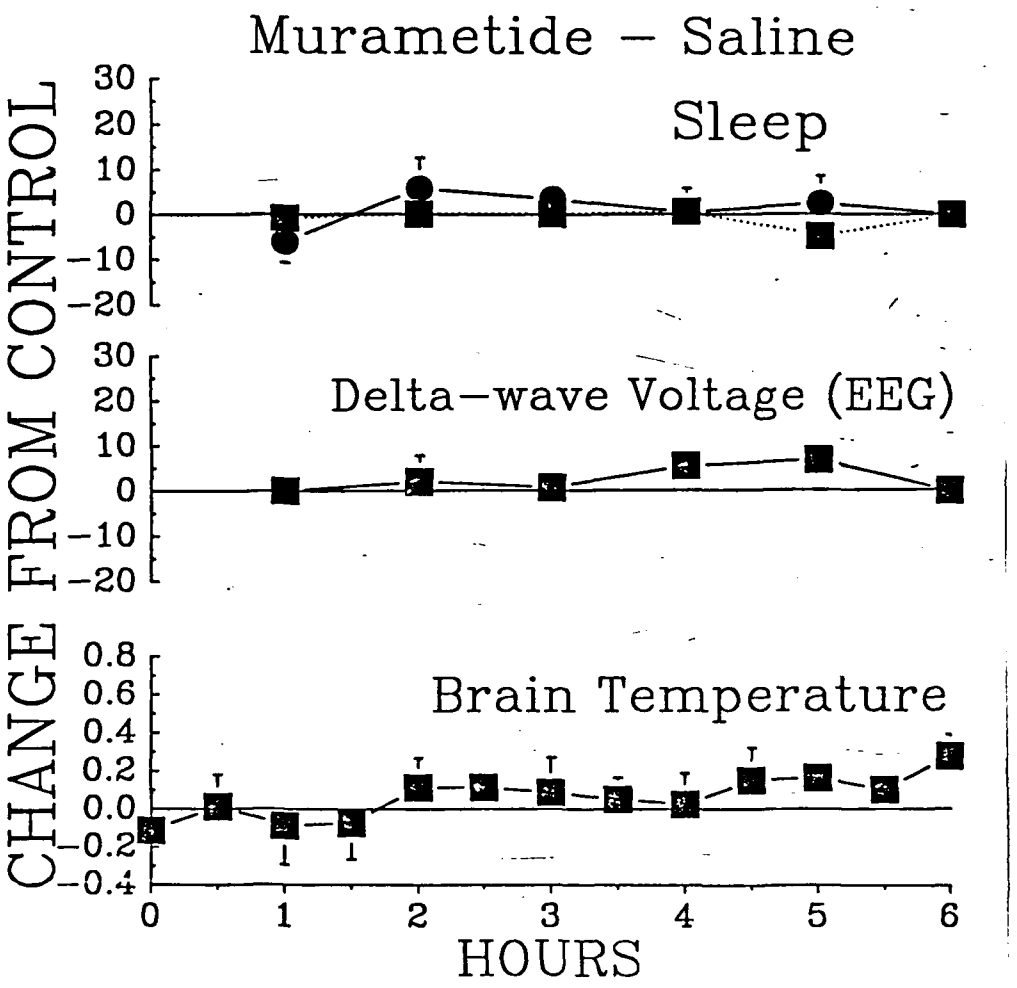

Fig. 1. Effects of IV injection of murametide on slow-wave sleep (SWS) (●——●), REMS (■---■), EEG DWA, and T_{br}. Values represent mean differences of experimental-control (saline) values (zero line). Ordinates for sleep and EEG DWA show differences of percentages (exper.-control), while the ordinate for T_{br} shows temperature differences (exper.-control, °C).n = 8 for all plots; error bars are standard error of the mean.

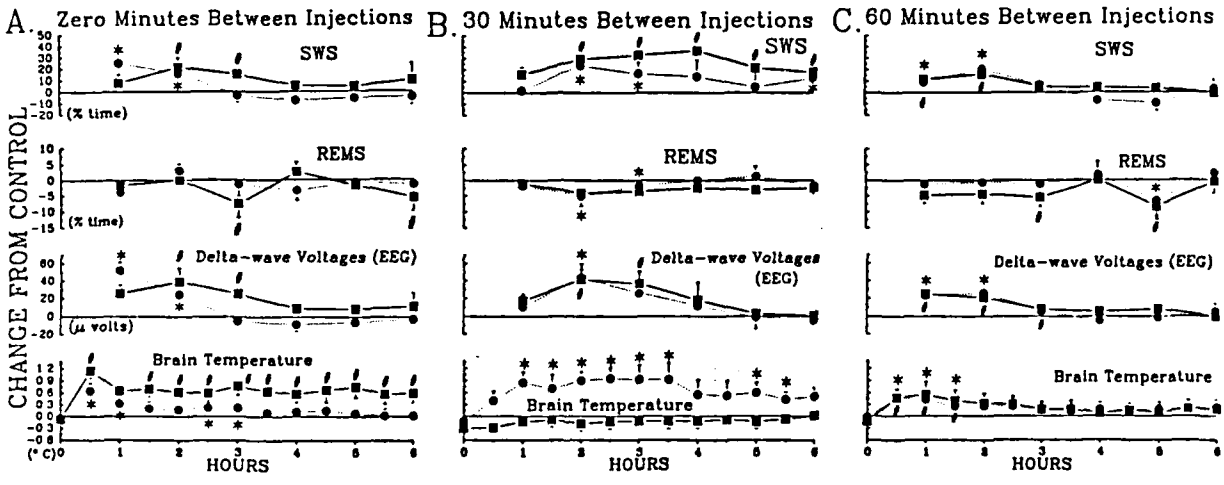

Fig. 2. Effects of murametide or rIL1β on SWS, REMS, EEG DWA, and T_{br}. Values represent saline + rIL1β injection (●---●) or murametide + rIL1β (■—■) injection sequences. Values are mean differences of experimental-control values (zero line). Ordinates for sleep and EEG delta wave amplitudes show differences of percentages (exper.-control), while the ordinate for T_{br} shows temperature differences (exper.-control, °C). n = 4 for graphs A and C (rIL1β, lot #1); n = 4 for graph B (rIL1β, lot #2) rIL1β + saline plots; n = 8 for graph B murametide + rIL1β plots. Error bars are standard error of the mean. * = significant difference from control, Wilcoxon Matched Pairs test ($p < 0.05$).

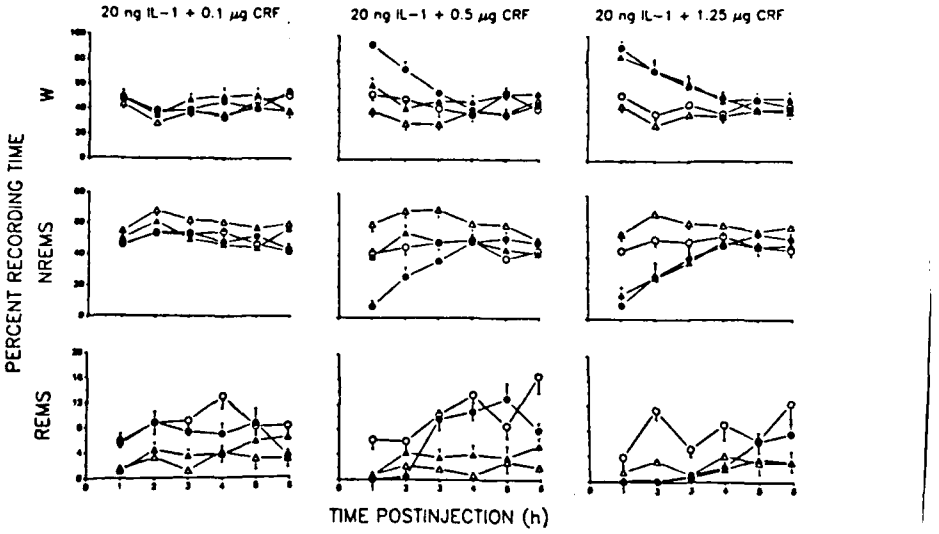

Fig. 3. Effects of 0.1 (Group 1, left panel), 0.5 (Group 2, middle panel), and 1.25 μg (Group 3, right panel) CRF, 20 ng IL1, and 20 ng IL1 + CRF on rabbit sleep-wake activity. Each point, percent time spent in vigilance states (W, NREMS, REMS) as means ± SE of eight rabbits (except Group 2, n = 7) after ICV injection of aCSF (open circles), CRF (closed circles), IL1 (open triangles), or IL1 + CRF (closed triangles). CRF dose-dependently reduced NREMS and REMS, and IL1-induced excess NREMS.

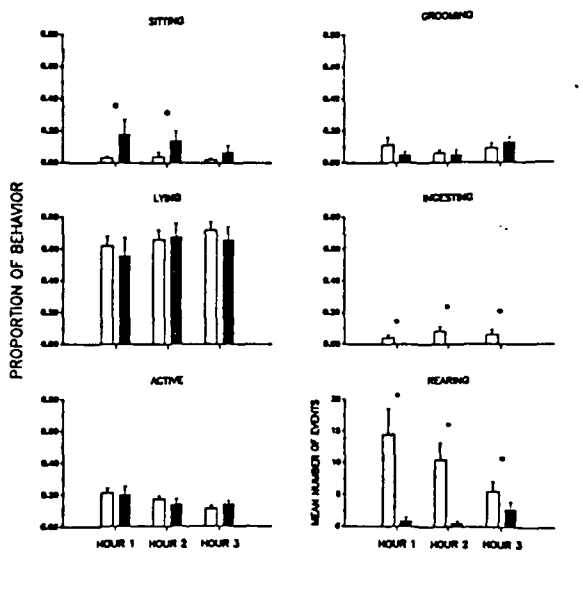

Fig. 4. Rabbit behavior after ICV injection of aCSF (open bar) or 1.25 μg CRF (closed bar). Proportions of behavior are means ± SE for eight rabbits. REARING was scored as a discrete event and is presented as mean ± SE number of events (see METHODS for behavioral descriptions). *significant differences ($p < 0.05$, Wilcoxon test).

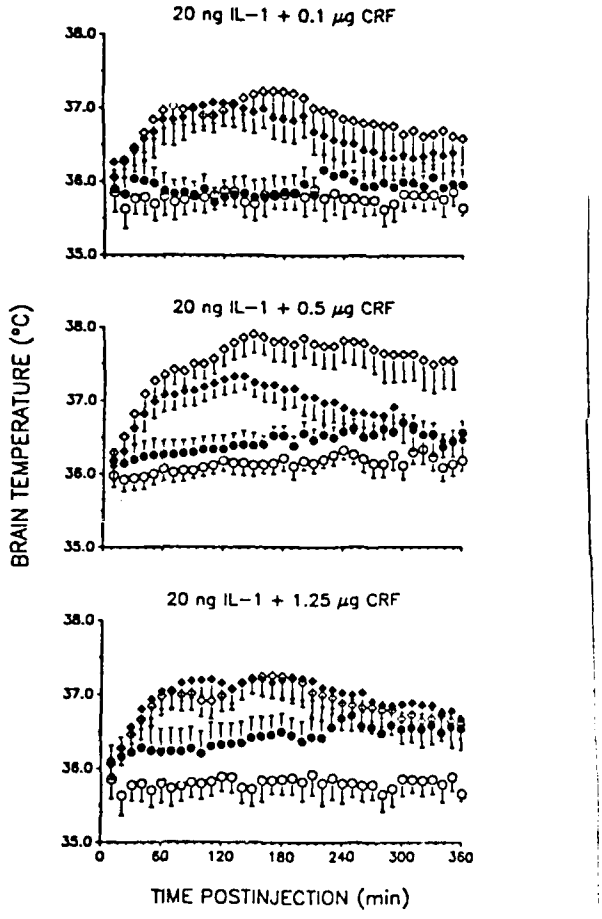

Fig. 5. Time courses of T_{br} after ICV injection of aCSF (open circles), CRF (solid circles; Group 1, top panel, 0.1 μg; Group 2, middle panel, 0.5 μg; Group 3, bottom panel, 1.25 μg), IL1 (open triangles), or IL1 + CRF (solid triangles). A double injection protocol was followed (see METHODS). Therefore, time 0 immediately follows the second injection. Values are means ± SE for eight rabbits (except middle panel, n = 7).

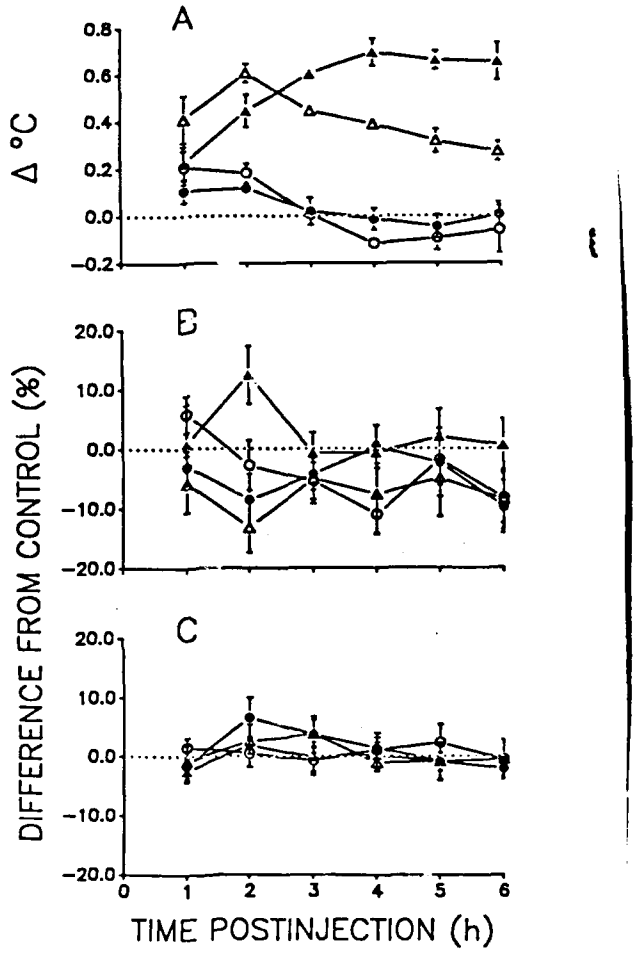

Fig. 6. Effects of IL6 (interferonβ_2/B-cell-stimulating factor/hybridoma growth factor) on rabbit sleep-wake activity and T_{br}. (A) T_{br} expressed as change relative to control values (represented by dotted zero line). (B) Change in NREMS relative to controls (represented by dotted zero line) expressed as percent recording time. (C) Change in REMS relative to controls (represented by dotted zero line) expressed as percent recording time. Each point represents the mean ± SE for six rabbits (except panel A; n = 5 due to one thermistor malfunction). Symbols are as follows: 20 ng IL6 (open circles), 40 ng IL6 (closed circles), 80 ng IL6 (open triangles), and 200 ng IL6 (closed triangles).

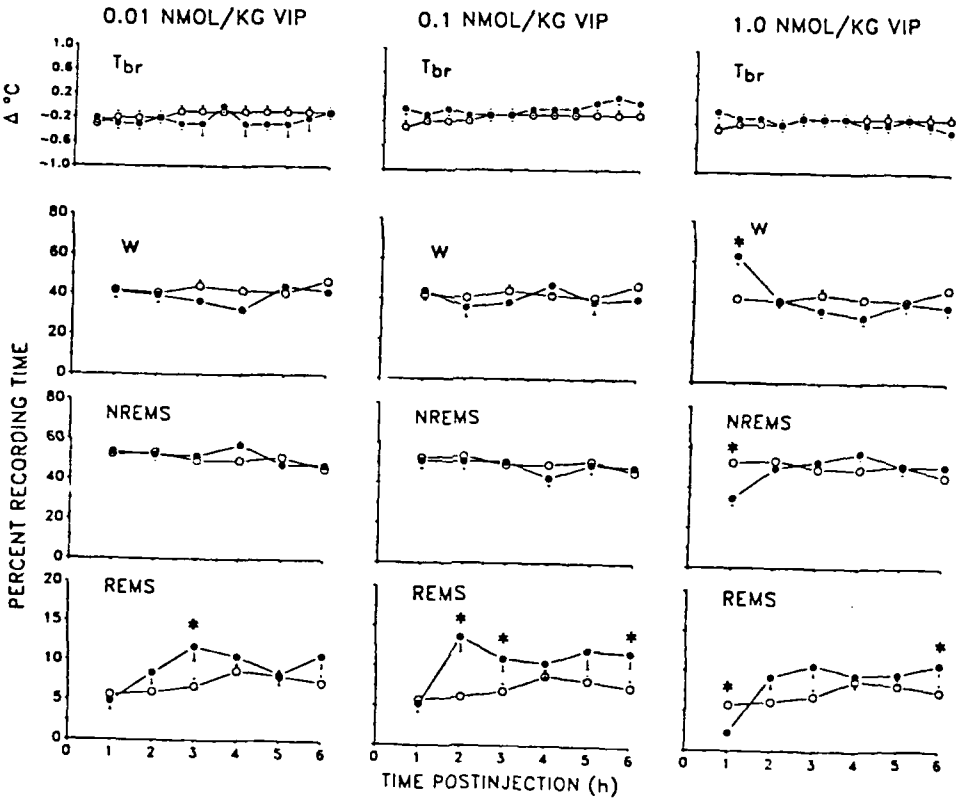

Fig. 7. Effects of ICV injection of VIP (0.01, 0.1, and 1.0 nmol/kg; n = 13 for each) on T_{br} and sleep-wake activity in rabbits. Mean percentages (± SE) of vigilance states (W, NREMS, REMS) are shown for each postinjection hour after ICV administration of aCSF (open symbols) and VIP (closed symbols). T_{br} is depicted in 30-min intervals as changes from the initial value. Asterisks mark significant differences ($p < 0.05$) between baseline and test values (Wilcoxon matched-pairs signed-rank test [two-tailed] for those cases where Friedman's test for k-related samples indicated significant effects across the 6-h recording period).

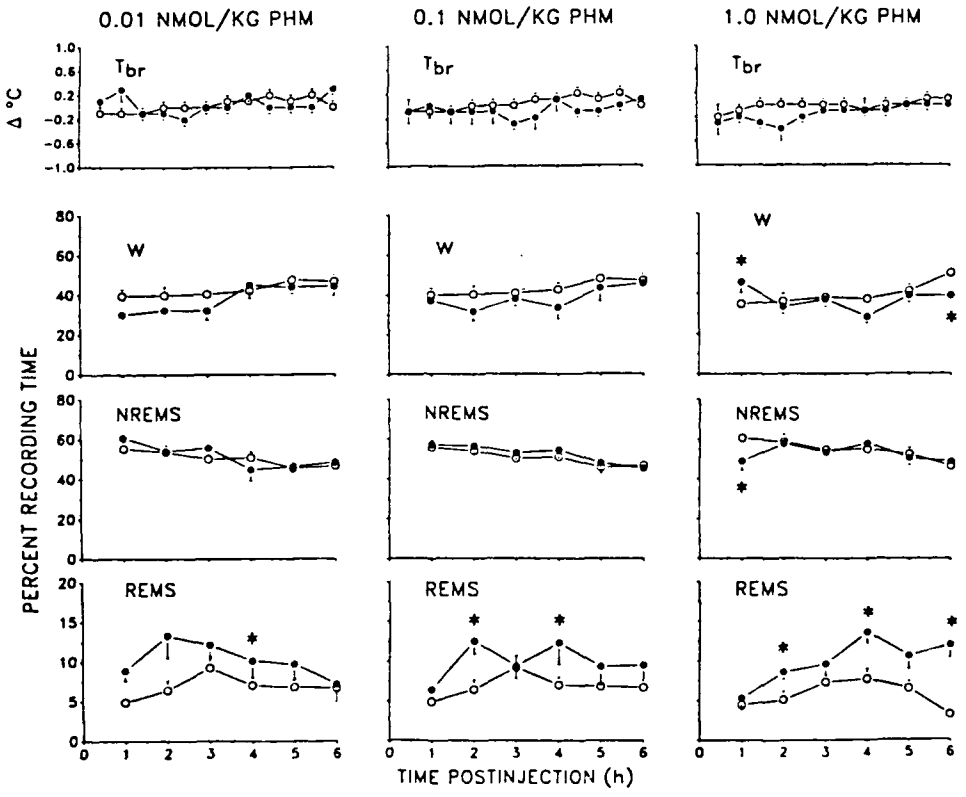

Fig. 8. Effects of ICV injection of PHM (0.01 and 0.1 nmol/kg [n = 8], and 1.0 nmol/kg [n = 12]) on T_{br} and sleep-wake activity in rabbits. Mean percentages (± SE) of vigilance states (W, NREMS, REMS) are shown for each postinjection hour after ICV administration of aCSF (open symbols) and PHM (closed symbols). T_{br} is depicted in 30-min intervals as changes from the initial value. Asterisks mark significant differences ($p < 0.05$) between baseline and test values (Wilcoxon matched-pairs signed-rank test [two-tailed] for those cases where Friedman's test for k-related samples indicated significant effects across the 6-h recording period).

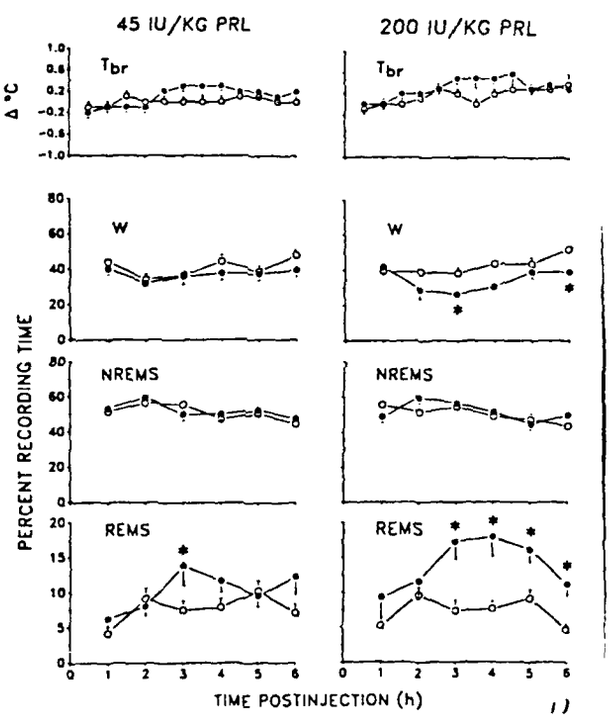

Fig. 9. Effects of SC injection of PRL (ovine PRL, 45 IU/kg [n = 14], and 200 IU/kg [n = 8]) on T_{br} and sleep-wake activity in rabbits (closed symbols). The vehicle was administered before baseline recording (open symbols). T_{br} is depicted in 30-min intervals as changes from the initial value. Asterisks mark significant differences ($p < 0.05$) between baseline and test values (Wilcoxon matched-pairs signed-rank test [two-tailed] for those cases where Friedman's test for k-related samples indicated significant effects across the 6-h recording period).

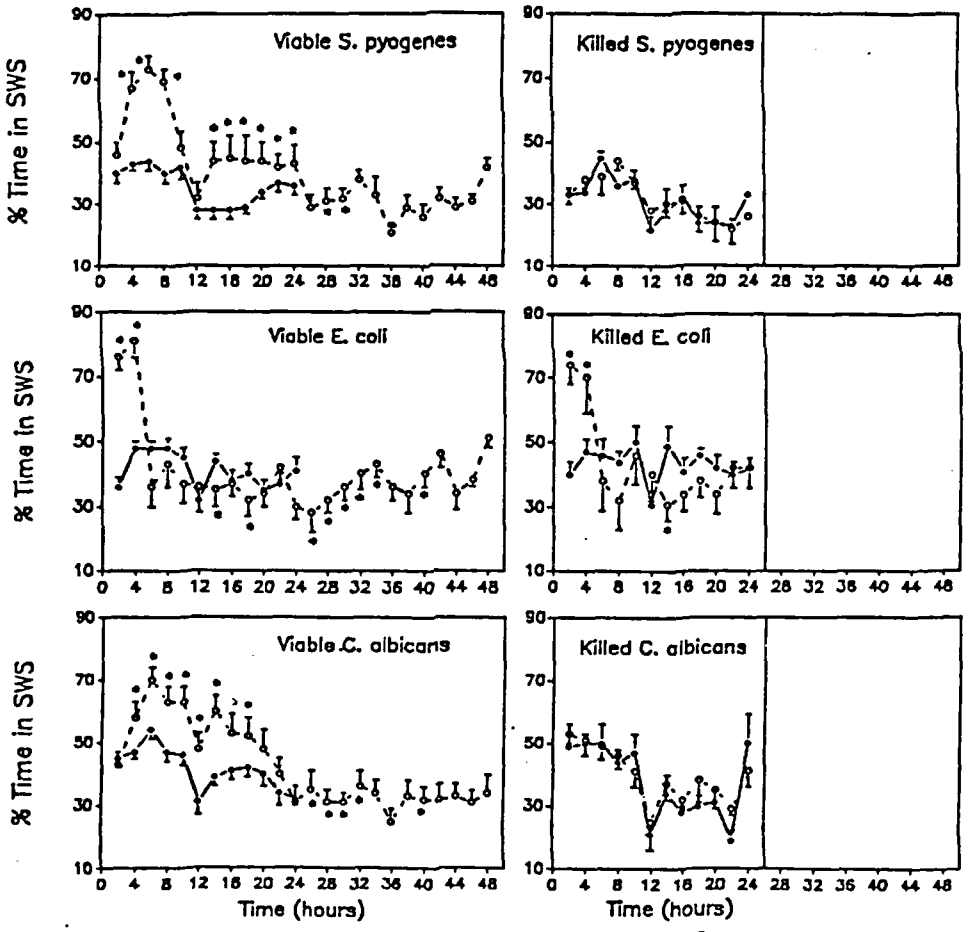

Fig. 10. SWS in rabbits after microbial challenge. SWS was monitored for 24 h prior to (●——●) and 48 h after (o---o) the IV inoculation of rabbits with viable microbial organisms or with the same dose of heat-killed organisms. The following agents, doses, and numbers of animals were used: viable S. pyogenes, 1×10^9 CFU, n = 10; viable E. coli, 9×10^7 CFU, n = 12; viable C. albicans, 4×10^7 CFU, n = 12; heat-killed S. pyogenes, n = 4; heat-killed E. coli, n = 6; heat-killed C. albicans, n = 4. Lights were off during hours 11-23 and 35-47. Data points represent the mean ± S.E.M. of values obtained from each rabbit during the preceding 2-h period. ANOVA: F = 9.05, 221 d. f., $p < 0.0001$; *$p < 0.05$ relative to corresponding baseline values.

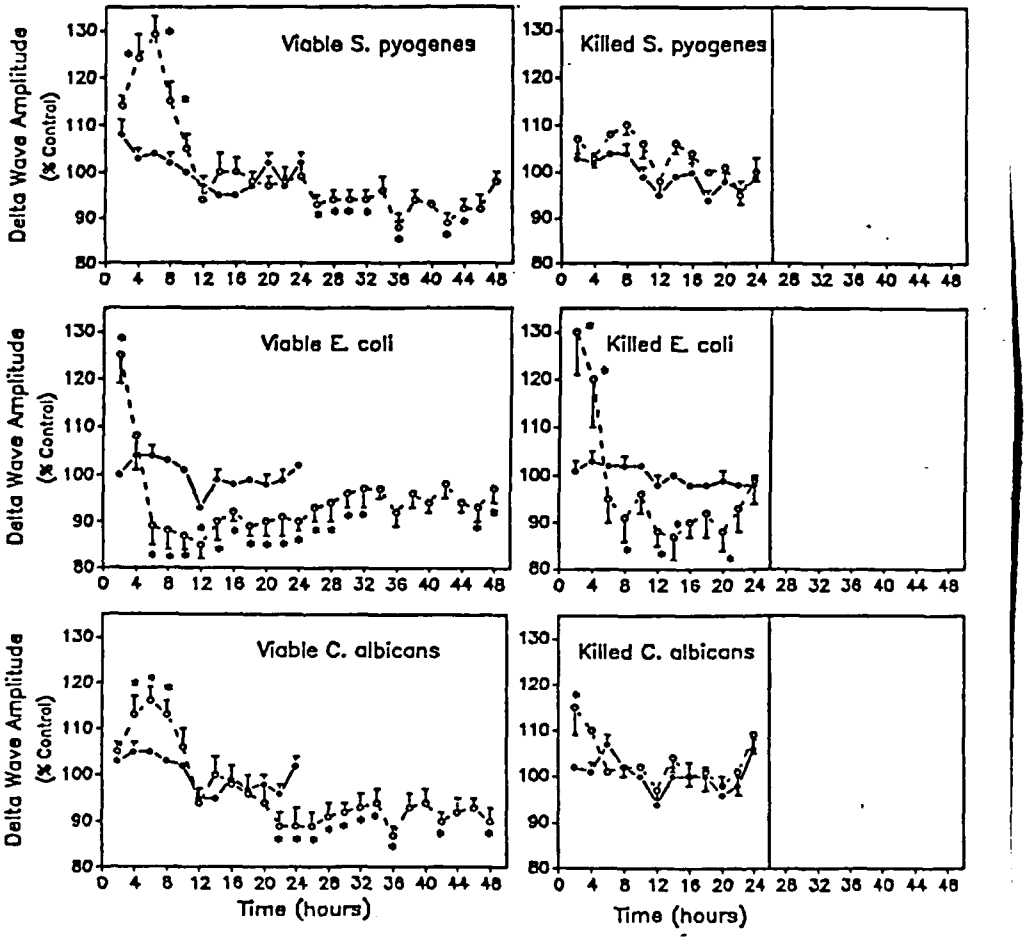

Fig. 11. DWA in rabbits after microbial challenge. DWA during SWS were monitored for 24 h prior to (●—●) and 48 h after (o---o) the IV inoculation of rabbits with viable or heat-killed microbial organisms, as described in Fig. 10. ANOVA: $F = 7.72$, 221 d. f., $p < 0.0001$; *$p < 0.05$ relative to the corresponding baseline values.

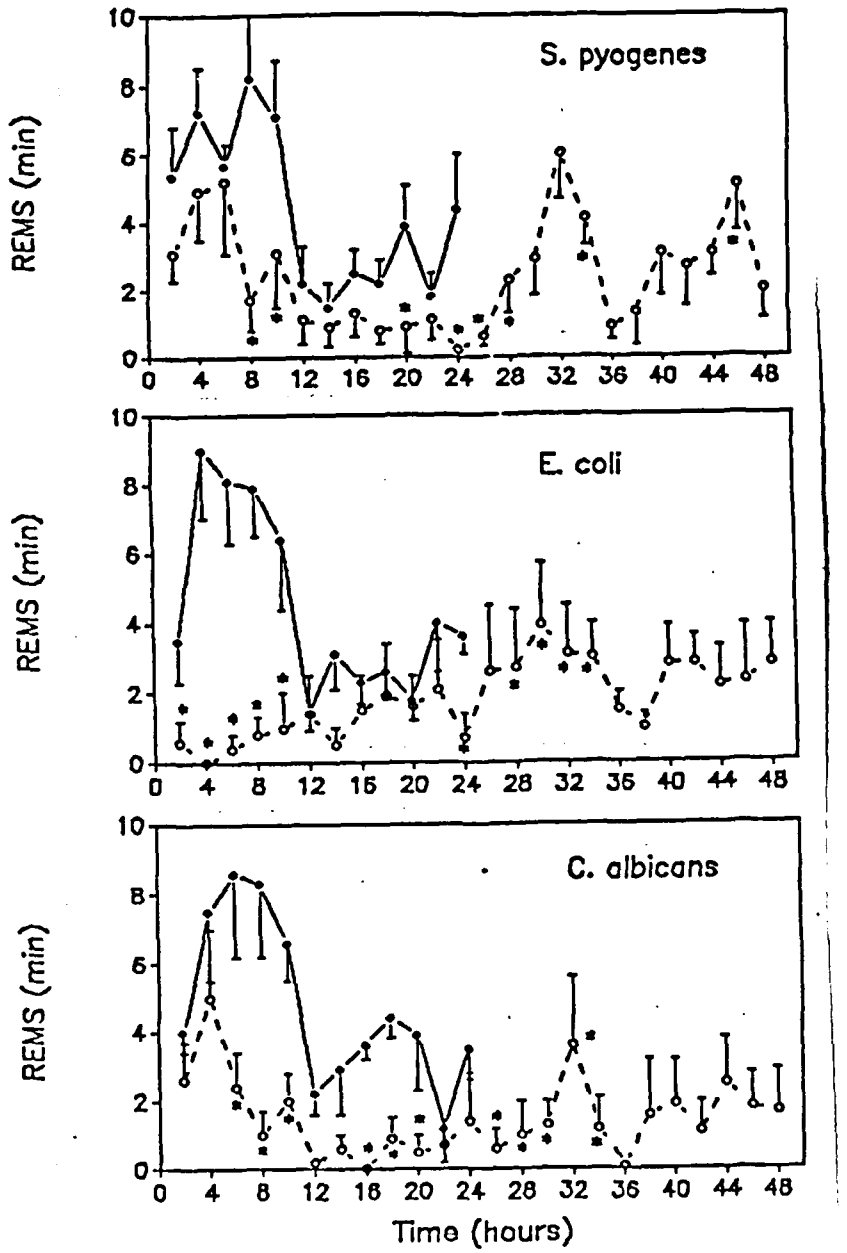

Fig. 12. REMS in rabbits after microbial challenge. REMS was monitored for 24 h prior to (o---o) and 48 after (o---o) the IV inoculation of rabbits with viable microbial organisms, as described in Fig. 10. Numbers of rabbits tested were: S. pyogenes, n = 7; E. coli, n = 8; C. albicans, n = 4. ANOVA: $F = 4.59$, 124 d. f., $p < 0.0001$; *$p < 0.05$ relative to the corresponding baseline values.

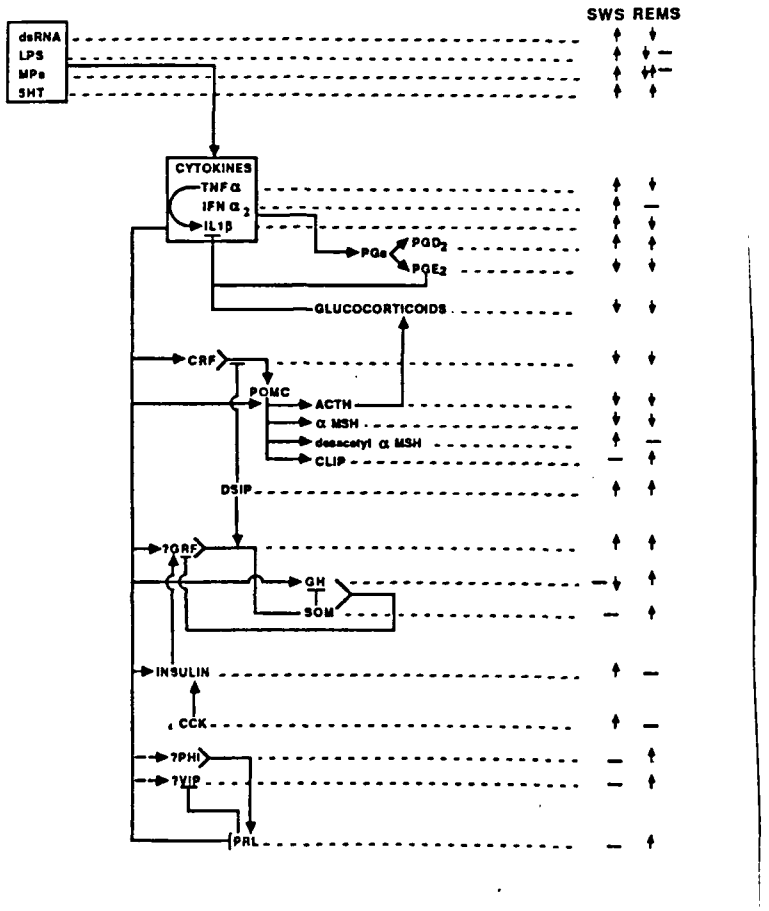

Fig. 13. Sleep activational system showing possible interaction between putative sleep factors and effects of those sleep factors on NREMS and REMS. dsRNA = double-stranded ribonucleic acid; POMC - proopiomelanocorticotropin; CLIP - corticotropin-like intermediate lobe peptide; DSIP - delta sleep-inducing peptide; SOM - somatostatin; CCK - cholecystokinin; PHI - peptide histidine isoleucine. See Reference 84 for original references concerning the sleep effects of these compounds. Left lines, → indicates stimulation and - indicates inhibition. Arrows on right indicate sleep effects; ↑ indicates increases; ↓ decreases, — no effect.

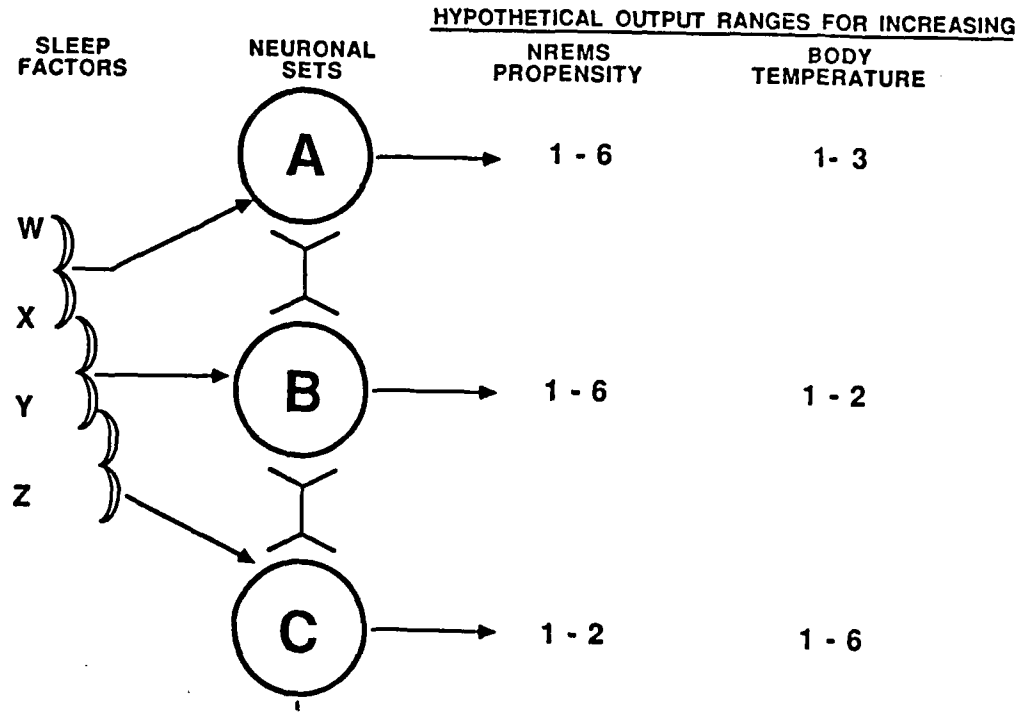

Fig. 14. Illustration of how sleep factors with multiple biological activities may selectively alter sleep.

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