Ann. Rev. Pharmacol. Toxicol. 1978. 18:563–79

THE NEUROPHARMACOLOGY OF SLEEP AND WAKEFULNESS

✦6729

J. Christian Gillin,[1] *Wallace B. Mendelson, Natarajan Sitaram, and Richard Jed Wyatt*

Laboratory of Clinical Psychopharmacology and Biological Psychiatry Branch, National Institute of Mental Health, Washington, DC and Bethesda, Maryland 20014

INTRODUCTION

Attempts to understand the physiology of sleep and wakefulness have been governed, on the one hand, by the specific phenomena that the investigator has chosen to explain, and, on the other, by the concepts and techniques available for studying the central nervous system. Prior to the discovery of rapid eye movement (REM) sleep in 1953, and its association with dreaming, investigators had only to explain sleep and wakefulness and the oscillation between the two states. Thus, investigators of "wet" physiology looked for "hypnotoxins" which accumulated during wakefulness and which were presumably dissipated during sleep (1). Similarly, investigators of "dry" physiology explained sleep as a passive state resulting from inactivity of the reticular activating system (2).

The discovery that consciousness consists of three major states (REM, wakefulness, and nonREM) complicated but not not obviate these older approaches. It became necessary, however, to account not only for the physiology of each state, but also for their orderly sequence (that is, sleep normally begins with the nonREM phase and consists of the well-known nonREM-REM cycle). Thus, more recent biochemical models, such as that of Jouvet (2), postulates that the mechanism responsible for nonREM sleep "primes" and "triggers" REM sleep. Similarly, the neurophysiological model of Hobson & McCarley (3) suggests that nonREM and REM sleep result from the reciprocal interaction of two anatomically distinct neuronal groups. Physiological models that account for this normal, orderly sequence (wakefulness → nonREM → REM) must be flexible enough, however, to account ultimately for the expected deviation (wakefulness → REM → nonREM) which is seen in certain pathological conditions (narcolepsy, psychotic depression), following REM sleep deprivation, and in "phase shift" studies.

[1]Address correspondence to J. C. Gillin, MD, NIH 10/3N224, Bethesda, Maryland 20014.

0362-1642/78/0415-0563$01.00

Considerable attention has also focused also upon physiological characteristics specific to each state of consciousness. The electroencephalogram (EEG) is of particular importance in defining the states. During human wakefulness, the EEG varies from low voltage, fast activity to synchronized alpha activity. During REM sleep, the EEG is also activated, consisting of a low voltage, mixed frequency pattern. In man and primates, four stages of nonREM sleep can be defined on the basis of the EEG: Stage I, a brief transitional phase with a mixed frequency, low voltage EEG; Stage II, defined by K-complexes and sleep spindles; and Stages III and IV (together referred to as delta sleep) defined by moderate and large number of high voltage, slow waves, respectively. In cats and rodents, two stages of nonREM sleep may be defined by the presence or absence of high voltage slow waves. Very regular theta waves in the hippocampus; monophasic waves in pons, geniculate body, and occipital cortex [pontine geniculate occipital (PGO) spikes]; and atonia of the major muscles [electromyogram (EMG) suppression] are also characteristic of REM sleep. The physiological events of REM sleep may be classified as tonic or phasic, depending upon whether or not they occur continuously throughout the REM period (the EEG, EMG suppression) or periodically (eye movements, PGO spikes).

The conceptual and technical approaches to the study of sleep have been dominated for the past 15 years by concern with the biogenic amines [serotonin (5-HT), dopamine (DA), and norepinephrine (NE)] and, to a lesser extent, with acetylcholine (ACh). Progress in understanding the physiological role of biogenic amines was made possible by the development of histofluorescent techniques that permitted anatomic localization of specific pathways, and by the availability of numerous pharmacologic agents capable of altering them.

The major research strategies have been (*a*) to determine changes produced by experimental manipulation of specific neurotransmitter systems and (*b*) to correlate activity within a specific system with physiological states of consciousness. Additional techniques have included the search for transferable "sleep factors" and correlations between the state of consciousness and circadian factors.

That sleep researchers have focused so extensively upon biogenic amines and ACh is a reflection of the intellectual debt we owe Michel Jouvet and his colleagues (2), who pioneered many of the concepts and techniques reviewed here. They suggested that nonREM sleep is initiated by the synaptic release of 5-HT from neurons originating in the rostral raphe nuclei in the brain stem. Furthermore, REM sleep is initiated by the release of 5HT from neurons originating in the caudal raphe nuclei, and wakefulness and cortical arousal depend on NE-containing neurons of the anterior locus coeruleus, DA-containing neurons in the mesencephalic reticular formation, and ACh neurons in the cortex. Moreover, "executive" mechanisms responsible for REM sleep involve catecholeamines (CA) and, possibly, ACh. NE-containing neurons within the caudal third of the locus coeruleus are responsible for EMG suppression of REM sleep, whereas those of the medial one third of the locus coeruleus function as the pacemaker for PGO activity and are responsible for both the phasic and tonic ascending components of REM sleep.

Many reviews have appeared in recent years (2–8). In this paper we briefly review the role of the monoamines and ACh, and, in addition, the possibility of so-called sleep factors and activating factors which may be involved in sleep.

SEROTONIN

Experimental Manipulation of the Serotonergic System

Since Brodie & Shore (9) first suggested that serotonin (5-HT) partially controls rest and activity, several drugs, particularly reserpine and parachlorophenylalanine (PCPA), have been used to decrease brain 5-HT concentrations and to study sleep. Reserpine decreases the concentration not only of 5-HT but of NE and DA. In the cat and rat it suppressed both nonREM and REM sleep (2). The cat also showed PGO spikes during waking (2, 10). When the 5-HT precursor, 5-hydroxytryptophan (5-HTP), was given to the reserpinized cat, nonREM sleep reappeared without PGO spiking during wakefulness and nonREM sleep.

PCPA decreases 5-HT concentration by inhibiting tryptophan hydroxylase, the rate-limiting enzymatic step in 5-HT synthesis. Single high dosages of PCPA produced total loss of sleep, lasting up to a week in the cat and monkey (2) [Chap. 2 in (6)]. PGO spikes were observed in nonREM and waking; administration of 5-HTP blocked the appearance of PGO spikes during wakefulness and nonREM. When PCPA was given chronically, however, nonREM and REM sleep gradually reappeared at near normal levels despite markedly low brain 5-HT concentrations; moreover, PGO spikes continued during wakefulness and nonREM sleep (11).

Other investigators have not observed all of these sleep changes induced by PCPA. Ursin observed only a reduction in the slow wave stages of nonREM sleep in the cat (12). Similarly, Weitzman et al (13) reported reduced slow wave stages of nonREM sleep in monkeys shortly after administration of PCPA. In humans treated with PCPA for medical conditions, Wyatt (8) observed little change in nonREM sleep (these patients had few slow waves in their sleep) but a marked decrease in REM sleep; 5-HTP restored REM sleep in the PCPA-treated patient, but tryptophan did not. In contrast, Rechtschaffen et al (14) reported that PCPA did not affect the sleep of the rat; this conclusion was reached, however, before their more recent interest in nonREM sleep with and without delta waves.

Methysergide, which appears to block the serotonin receptors, decreased REM sleep in man (15) and rabbit (16). In man, methysergide did not change total delta sleep; total nonREM was slightly increased.

When lesions of dorsal and medial raphe nuclei were used to decrease 5-HT in the adult cat, total sleep initially decreased, particularly nonREM (2); however, when the animals are monitored over a longer period of time, their sleep tended to return toward normal (7).

Along these lines, Adrien et al (17) lesioned the raphe dorsalis and centralis in newborn rats. Even though brain 5-HT concentration dropped to 5–10% of control, when the animals were studied at three weeks their sleep was unaltered.

Brain 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), may also be decreased by intraventricular administration of 5,6-dihydroxytryptamine which destroys serotonergic nerve terminals. REM sleep disappeared initially and remained at about 50% of normal over the next two weeks (18).

In a recent experiment in our laboratory, E. Moja, W. B. Mendelson, J. C. Gillin, and R. J. Wyatt were able to reduce brain 5-HT concentration to 35% and 5-HIAA to 50% of normal in rats given a tryptophan-deficient, amino acid diet. Although

motor activity and nonREM did not change, both REM sleep and the percentage of REM decreased significantly.

Application of 5-HT directly to the area postrema, which lacks a blood-brain barrier, increased synchronization of the EEG in paralyzed cats (19). Administration of the biosynthetic precursor of serotonin, tryptophan (Trp), increased slow-wave sleep in man and/or decreased latency of sleep onset (8, 20). The effect of Trp on total sleep time has been inconsistent [(6) Chap. 2]. In contrast, administration of 5-HTP in low doses did not affect slow-wave sleep in normal man but did increase REM sleep (8). High doses of 5-HTP suppressed REM sleep in neurologic and schizophrenic patients; in the latter group, very high doses (6 g/day) actually reduced total sleep time; furthermore, rapid withdrawal produced loss of sleep for about one week (J. C. Gillin, unpublished data). Administration of lower doses of 5-HTP has not generally increased total sleep [(6) Chap. 2]; exceptions included the administration of 5-HTP to reserpinized or PCPA-treated cats and to two individual human patients with marked hyposomnia (21, 22).

Electrical stimulation of the raphe was not reported to increase total sleep (23) but may, in fact, have produced arousal and suppression of PGO spikes during REM sleep (24).

Correlations Between 5-HT Activity and Sleep

Various measurements have been made of 5-HT and 5-HIAA concentrations during sleep. Increased ventricular fluid 5-HIAA during nonREM sleep as compared with wakefulness was reported in cats (25) and man (26), whereas decreased brain 5-HT was reported in cats (27) sacrificed during nonREM sleep. These results suggest increased turnover of 5-HT during nonREM sleep. Perhaps in contrast, however, hippocampal 5-HT was increased in cats during nonREM (28).

Single-cell recordings have also been made in the dorsal raphe nucleus. These neurons were most active in wakefulness and least during REM; moreover, many units ceased firing just before and in temporal association with PGO spikes (29).

CATECHOLAMINES

Experimental Manipulations of Catecholaminergic Activity

A variety of pharmacological compounds have been given to study the effects of increased DA and/or NE activity: L-dihydroxyphenylethylamine (L-dopa, the biosynthetic precursor of DA and NE) (8) [(6) Chap. 2], dextro- and levo-amphetamine (30), cocaine (31), piribedil (ET495, a DA agonist) (R. Gerner, J. C. Gillin, and R. M. Post, unpublished data), methylphenidate (32), and clonidine (33). All these agents tended to decrease total REM sleep time when initially administered. It is interesting that agents that increased DA activity relatively more than NE (L-dopa in high doses, *d*-amphetamine, methylphenidate, cocaine) decreased total sleep as well. The relatively pure NE agonist clonidine did not seem to have this effect.

With chronic administration of amphetamine and methylphenidate, tolerance to the sleep effects apparently developed because both hyperactive children and am-

phetamine addicts receiving chronically administered stimulants had relatively normal sleep patterns (32, 34). Interestingly, during withdrawal, amphetamine addicts showed increased total sleep and REM sleep (34). This is one of the few pharmacological manipulations that have been shown to markedly increase total sleep time.

The effects of CA antagonists have also been studied. Acute administration of reserpine (in relatively low doses) (35, 36) and α-methylparatyrosine (AMPT) [(6) Chap. 2] (8, 37), which deplete both DA and NE by enhancing release and blocking synthesis, respectively, appeared to increase REM sleep. Chronic administration with reserpine, but not with AMPT, also increased REM sleep. (It should be noted that both may also influence other amines.) α-Adrenergic receptor blockers such as thymoxamine (38) and phenoxybenzamine (39) have also been reported to induce REM sleep in normal volunteers and rats, respectively. DA receptor blockers, such as pimozide (40) and chlorpromazine (41), have not generally been reported to have robust effects on sleep.

In animal studies, large doses of reserpine were reported to decrease both REM and nonREM (2); administration of L-dopa to these animals restored REM sleep, while 5-HTP restored nonREM sleep. More recently, however, Brooks & Gershon reported that L-dopa similarly produced arousal in the reserpinized cat (10).

The CA area examined most closely by the lesion technique is the locus coeruleus (LC), comprised of NE-containing neurons in the dorsolateral pontine tegmentum. Following initial lesion studies of the locus coeruleus, REM sleep was reported to decrease in association with depletion of brain NE (2). Moreover, lesions of the sublocus coeruleus abolished the muscle atonia of REM sleep, thus permitting cats to ambulate while apparently in REM sleep, a finding subsequently replicated by others (42, 43). In a more recent study, Jones et al (43), however, found that lesions of the locus coeruleus produced no change in the total amount of REM sleep or wakefulness, although they noted fewer PGO spikes. Furthermore, 6-hydroxydopamine–induced lesions of CA neurons had little effect upon sleep (44). Lesion studies have also been oriented to the DA system. Lesions of the ventral mesencephalic tegmentum (including the substantia nigra and lemniscus medialis) did not change EEG waking but did produce hypertonus and lack of behavioral arousal (45). These results suggest that DA neurons might be involved in the maintenance of behavioral (but not EEG) wakefulness.

Spontaneous Changes in Noradrenergic Activity

Observations of single NE neurons in the locus coeruleus suggest the presence of two types of neurons. As reported by Hobson & McCarley (3), "D-on" cells fired progressively more rapidly during waking, nonREM, and REM sleep respectively. "D-off" cells had the reverse pattern. About 60% of locus coeruleus cells sampled by Hobson et al (3) were "D-off" cells, whereas Chu & Bloom (46) found that only about one quarter were of this type. Hobson and his associates suggest that the "D-off" cells fire in a reciprocal relationship with the cholinergic cells of the gigantocellular tegmental field or FTG of Berman (3). The latter cells increased firing rate immediately prior to the cortical activation of REM sleep, and, during REM sleep, increased firing rate further when PGO spikes occurred; the "D-off" NE cells of the

locus coeruleus were postulated to decrease their firing rate at the time that the FTG cells increased their rate. These observations are the basis for one model of the control system which might regulate the REM-nonREM cycle. Hobson et al (3) suggest that cells from FTG and locus coeruleus (LC) send fibers to each other and also back to the parent nucleus. Fibers from the locus coeruleus "D-off" cells are postulated to be inhibitory to themselves and to the giant cells of the FTG system. Conversely, the FTG cells are postulated to be facilitatory to themselves and to the locus coeruleus. In contrast to the hypothesis originally put forth by Jouvet (see Introduction), this hypothesis suggests that NE neurons, or at least the "D-off" cells of LC, inhibit REM sleep. The model is discussed further in the next section on ACh.

Evidence of CA activity may also be derived from examination of metabolites in the cerebrospinal fluid and urine during sleep. Wyatt et al (47) reported that both 3-methoxy-4-hydroxyphenylglycol (MHPG), a major metabolite of NE, and homovanillic acid (HVA), derived from DA, were higher during REM than in nonREM sleep in man. Since these data were derived from patients with severe dementia, interpretations must be made with caution. In cats sacrified during REM sleep, the NE concentration in cerebral cortex was significantly decreased from values obtained from nonREM sleep (27). This also might be consistent with reports of increased firing of some NE neurons during REM sleep (3, 46).

Postprobenecid HVA levels in the CSF were positively related to mean duration of REM sleep episodes in hypersomnic and narcoleptic patients and were higher in hypersomnia patients with excessive REM than in narcoleptics or "mixed hypersomniacs" (48). (None of the three groups differed from control subjects, however.) Likewise, postprobenecid HVA levels were lowest in patients with hyposomnia characterized by greatly decreased total REM sleep and decreased mean duration of REM sleep episodes (49).

Urinary MHPG, a substantial proportion of which is derived from brain, was found to be inversely related to REM time and percentage of REM sleep and to be unrelated to total sleep or delta sleep in psychiatric patients (50).

ACETYLCHOLINE

Unlike norepinephrine or serotonin, the evidence implicating acetylcholine in the sleep-waking cycle is almost entirely indirect. Lesioning and stimulation techniques have not been applied to study ACh in sleep for the simple reason that "cholinergic tracts" in the brain have not yet been reliably mapped. In spite of the above shortcomings there is strong converging evidence that ACh plays a dominant role in the initiation of REM sleep and in the maintenance of cortical arousal. The evidence may be summarized as follows.

Experimental Manipulation of the Central Cholinergic System

Extensive studies by Hernandez-Peon (51), using microcrystals of ACh in the cat, led to the mapping of an organized cholinergic sleep system descending from the

temporal medial and basal cortex, and ascending from the spinal cord, both of which converged on a pontobulbar final common path. Direct transition from waking to REM sleep or very long-lasting REM sleep episodes (up to 60 min) have been obtained by local injections of ACh agonists (like ACh crystals, carbachol, oxotremorine) into the pontine reticular formation (52, 53), including the FTG (54). In addition, certain components of REM sleep such as atonia, PGO spiking, and EEG desynchronization were produced by local injection of carbachol into the dorsal anterior pontine tegmentum of cats (55).

Cholinergic agents seem to enhance REM sleep and/or produce arousal, whereas anticholinergic drugs suppress REM sleep. It is of interest that intravenous administration of physostigmine, an anticholinesterase agent, induced REM sleep in an *awake* cat only if it had been pretreated with reserpine or had been transected at the collicular or pontine level (56). If infused during nonREM sleep, however, it induced REM sleep in man (57, 58) and cats (59). This effect was found to be both dose- and time-dependent; 0.5 mg physostigmine, which induced REM when infused during the first nonREM period in man, produced wakefulness when administered during the first REM period or second nonREM period. A lower dose (0.25 mg), however, induced REM sleep without arousal when given during the second nonREM period (58). Furthermore, administration of physostigmine (1 mg) by *slow* i.v. drip beginning 35 min after sleep onset for 60 min accelerated the time of onset of both the first and second REM periods without altering their duration (J. C. Gillin, N. Sitaram, W. B. Mendelson, R. J. Wyatt, unpublished data) (Figure 1). This suggests that cholinergic mechanisms may control the onset rather than the duration of REM sleep; furthermore, once the early REM period was "moved forward," the later ones were also, suggesting that the onset of each REM period is governed by the preceding one. Physostigmine-induced REM periods were also associated with dreaming in man (N. Sitaram, A. Moore, and J. C. Gillin, 1977, unpublished data).

The question of whether REM induction and arousal results from central nicotinic, or muscarinic actions or both of ACh remains unresolved. The report by Domino et al (59) that the REM-enhancing actions of physostigmine and pilocarpine (a muscarinic agonist) were antagonized by atropine but not by methylatropine or nicotinic blockers, trimethidinium and mecamylamine, supports a central muscarinic action. On the other hand, both nicotine (59) itself and piperidine (4), an alicylic amine with nicotinic activity, induced REM sleep in cats. The central muscarinic blocker, scopolamine, and the ACh synthesis blocker, hemicholinium-3, consistently suppressed REM sleep (59, 60). Atropine, whose central action is about eight times less potent than scopolamine, acted inconsistently (61, 62). There are no studies on the action of the central nicotine blocker mecamylamine on the sleep-waking cycle.

Pompeiano and co-workers (63) have reported that intravenous infusion of physostigmine in precollicular decerebrate cats produced decerebrate rigidity and tonic depression of both flexor and extensor spinal reflexes. Superimposed on this tonic depression was a further phasic suppression associated with regular bursts of rapid

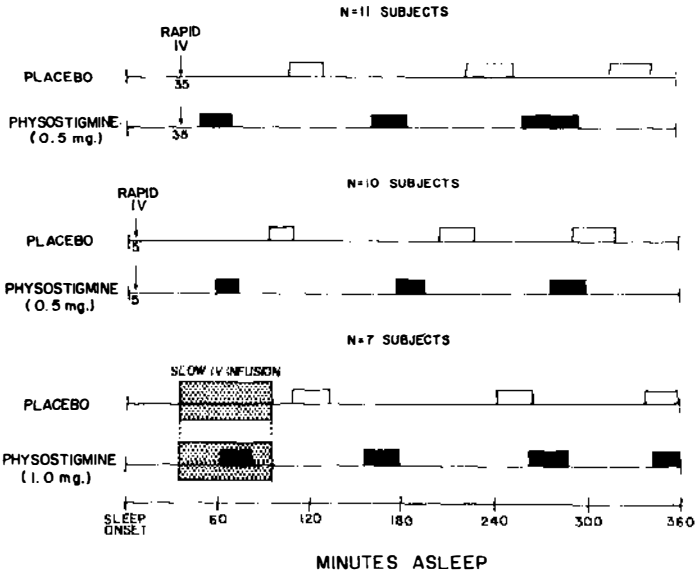

Figure 1 Physostigmine resets REM sleep (ultradian) rhythm in man. Bolus and slow infusions of physostigmine (0.5 mg and 1.0 mg, respectively) during the first nonREM period in man induce the onset of REM periods without altering their duration. Moving the first REM period forward also advances the onset of each of the subsequent REM periods (J. C. Gillin, N. Sitaram, W. B. Mendelson, R. J. Wyatt, unpublished data).

eye movements. The phasic EMG suppression and eye movements were presumably controlled by the medial vestibular nucleus and resulted from supraspinal descending inhibitory volleys from the pontine reticular formation.

A number of studies in cats have reported that physostigmine facilitated and atropine suppressed PGO spikes produced by reserpinization (64), REM deprivation (65, 66), and collicular and pontine transection (67). Both atropine and physostigmine are said to affect PGO waves that occur in bursts (Type II) and not those that appear singly (Type I).

Cholinergic Changes During Sleep

These studies, which rest on observations of physiological and neurochemical variations of acetylcholine systems under normal sleep and waking conditions, constitute some of the most reliable evidence linking ACh to sleep-wake cycle. Increased release of ACh during REM (as compared to nonREM) has been reported from the cortex (68) and striatum (69) in conscious moving cats and in ventricular perfusates of conscious dogs (70). ACh release was equal to or even greater than that during the waking state. Since REM sleep is accompanied, however, by increased blood circulation and temperature in the brain, it is not clear how specific the ACh release is to REM sleep.

As mentioned earlier, Hobson and co-workers (3), using single cell recording techniques in head-restrained cats, presented evidence that FTG neurons increased firing rates specifically and selectively during REM sleep. This was recently confirmed by Hoshino et al (71). Hobson et al suggest that these neurons are essential for REM sleep. Siegel & McGinty (72), however, have recently reported that FTG unit firing was selective not for REM sleep but rather for specific types of motor activation in unrestrained freely moving cats. Hobson et al (3) claim that those FTG neurons which generate REM sleep are cholinergic and cholinoceptive. That these neurons are cholinoceptive is supported by histochemical studies of acetylcholinesterase (AChE) activity (73) and by the report that iontophoretic administration of ACh chloride produced primarily excitation of these neurons (74). There is, however, no evidence to date that the FTG neurons are cholinergic. This would require demonstration of the presence of ACh, its precursor choline, and synthetic enzyme choline acetyltransferase (ChAT) inside the neuron and the release of ACh after presynaptic stimulation.

Richter & Crossland (75) showed that ACh content of whole rat brain increased significantly during sleep. Recently, Giacobini's group showed that piperidine increased significantly in content during dormancy in mice (76) and snails (77). Perfusion of piperidine into midbrain reticular formation of cats also induced REM sleep (4). The systematic study of ACh steady state and turnover rates in different parts of the brain during specific stages of normal sleep is in our opinion one of the most fruitful areas of future exploration.

The effects of total or partial sleep deprivation upon ACh have also been studied. REM sleep deprivation has been reported to produce a significant fall in brain ACh in the telencephalon (78, 79). Levels of ACh in the striatum were significantly enhanced (by 28%) after 10 days of REM deprivation in rats (80). Sagales & Domino (81), however, were not able to document any change in whole brain ACh content in REM-deprived mice. Tsuchiya et al (79) further reported that 24-hr total sleep deprivation, in contrast to REM deprivation, increased rat telencephalatic content of ACh. Scopolamine produced greater central anticholinergic effects (i.e. memory deficit, hallucinations, etc) in normal human subjects deprived of sleep for one night than in nondeprived controls (82). Conversely, physostigmine reversed the subjective effects of vigilance impairment of deprivation of one night's sleep in the subjects.

In summary, central cholinergic mechanisms seem to play a dominant role in the initiation of both tonic and phasic components of REM sleep. They may also play a direct or modulatory role in inducing EEG desynchronization (arousal) during waking.

SLEEP FACTORS

In 1913 Pieron (1) reported that CSF from donor dogs, deprived of sleep for 10 days or more, induced behavioral sleep in recipient dogs for several hours after intraventricular injection. Similar results were claimed twenty-five years later by Schnedorf & Ivy (83). They observed behavioral sleep in 9 of 20 recipient dogs injected

intracisternally with CSF (8 ml) from donor dogs which had been sleep deprived for 7 to 10 days; "sleep" was observed in 4 of 24 dogs receiving injections of CSF from control dogs not deprived of sleep. The sleep-inducing effect of CSF from experimentally sleep-deprived animals did not differ significantly from that of control animals, Chi-square test, performed by us. The "experimentally induced sleep" was not normal since it was accompanied by increased body temperature and CSF pressure.

Beginning in the mid 1960s, Pappenheimer and his associates (84, 85) reported that intraventricular infusion in rats of CSF (0.1 ml over 30 min) from sleep-deprived goats increased EEG slow-wave activity and decreased nocturnal motor activity in the first 6 hr as compared with infusions of CSF from control goats. Later studies indicated that the sleep-promoting factor (Factor S) would be extracted, partially purified, and concentrated from CSF and from acid-acetone extracts of brain stem and cortex of sleep-deprived goats and sheep; further, it was active in rabbits as well as rats. Sleep Factor S appears to be a peptide with a molecular weight of 350–500; it is inactivated by pronase. Although normal human or goat CSF had no effect in the rat activity assay after ultrafiltration and 20-fold concentration, a 50-fold concentration of normal human CSF did reduce nocturnal activity in rats by 54%, suggesting that Factor S is normally present in low concentrations in man. In addition, at least two excitatory peptides have been found in CSF (goat, human); the molecular weight appeared to be 500 to 10,000 (85). A single intraventricular injection of these peptides (less than 1 nmole) caused hyperactivity in rats for several days to several weeks.

In support of the hypothesis that CSF contains both sleep-inducing and activity-inducing substances, Sachs et al (86) compared the effects of rat CSF obtained from the dark, active period with that from the light, inactive period of the diurnal cycle. Intraventricularly administered CSF from light-exposed, inactive donor rats reduced motor activity in recipient rats during their dark-exposed active period. Conversely, CSF from dark-exposed active rats increased motor activity in recipient rats during their light-exposed inactive period. In contrast, Ringle & Herndon (87) failed to detect sleep-promoting properties for CSF from sleep-deprived rabbits when injected intraventricularly in rats, as measured by EEG patterns, ambulatory activity, and behavioral observations.

Like Pappenheimer and his associates, Nagasaki et al (88) reported sleep-promoting properties from brain extracts of rats who were kept awake by electric shock at the onset of nonREM sleep. When a pooled extract from six sleep-deprived rat brainstems was injected intraperitoneally into donor rats, decreased nocturnal locomotor activity and increased delta activity were observed about 12 hr later. No sleep-promoting activity was reported in brains of control rats. More recently, Nagasaki et al (89) reported that the brain extract of sleep-deprived rats significantly inhibited the spontaneous discharges of crayfish abdominal ganglion at low concentrations (10^{-10} mole/ml). Extracts from control rats not deprived of sleep were inhibitory at concentrations 1000-fold higher. Neither GABA nor 5-HT were shown to be responsible for the inhibitory effect of this brain extract from sleep-deprived animals.

Drucker-Colin and his colleagues (4) reported that perfusates of the midbrain reticular formation obtained from sleeping cats induced sleep when perfused into the midbrain or preoptic area of waking recipient cats; likewise, awake donor's perfusate tended to awaken sleeping animals. The active component of the perfusate has not been characterized. Furthermore, peak release of proteins from the midbrain reticular formation was highest during REM sleep.

In a series of studies beginning in the early 1960s, Monnier and his associates (90, 91) reported that delta waves could be induced in recipient rabbits with the systemic or intraventricular administration of a dialysate of cerebral venous blood obtained from donor rabbits who responded with increased cortical EEG slow-wave activity to electrical stimulation of the "sleep area" in ventromedial thalamus. Increased "sleep" in the recipient was measured by increased delta activity and decreased locomotor activity. No significant change developed in arterial blood or cerebrospinal fluid pressure, or respiration rate. Mild bradycardia accompanied the "delta sleep," as it does during physiological sleep.

More recently, Monnier et al (91) found that sleep factor delta was a nonpeptide with the sequence of Trp–Ala–Gly–Gly–Arp–Ala–Ser–Gly–Glu, which induces spindle and delta EEG activity in recipient rabbits.

In contrast to Pappenheimer's Sleep Factor S, the effects of which begin 2 hr after the infusion and last 5 or more hours, Monnier's sleep factor delta has effects that begin quickly after the onset of the infusion and end quickly after the end of the infusion.

The existence of bloodborne sleep factors has also been suggested by the observation that pairs of parabiotic rats display significantly higher synchronization rates of REM sleep than control pairs of rats who are attached by skin only (92). In contrast, evidence against the bloodborne hypnotoxin includes the observations of completely independent sleep patterns in human Siamese twins joined at the head (93) and in the dog with an extra head transplanted into its circulation (94).

DISCUSSION

Despite the considerable amount of research over the past 70 years, the neurochemical basis of sleep and wakefulness remains elusive. Both 5-HT and "sleep factors" have been hypothesized to initiate and maintain nonREM sleep. The data supporting this role for serotonin come primarily from studies with PCPA and raphe lesions. The data favoring sleep factors came originally from studies in which semipurified chemicals were isolated from sleep-deprived animals and administered to donor animals with a resulting increased slow activity and decreased motor activity.

Unfortunately for the theory that 5-HT plays a role in nonREM sleep, considerable evidence exists that fails to support it. Long-term depletion of brain 5-HT by either raphe lesions or the administration of PCPA has not been found to produce long-term loss of sleep. Moreover, not all investigators are in agreement that depletion of brain 5-HT by raphe lesions or pharmacologic agents necessarily reduces sleep time. Furthermore, electrical stimulation of raphe neurons or administration

of 5-HT precursors have generally failed to increase total sleep and, to the contrary, may cause arousal in some circumstances. Single-cell recordings from raphe neurons have not produced evidence that strongly favors a role for 5-HT neurons in the intiation or maintenance of nonREM sleep.

Much of the evidence does suggest, however, that 5-HT is involved in regulation of states of consciousness even if not in the role first formulated. Evidence from studies of raphe lesions, of pharmacologic depletion and repletion of brain 5HT, and of single-cell recordings support the hypothesis that 5-HT–containing neurons play a prominent role in the regulation of PGO spikes. It is also possible that 5-HT is one component in a system of balances, a homeostatic system possibly involving catecholamines and other neurotransmitters that regulate sleep-wakefulness states; this interpretation might be consistent with the following observations: (*a*) a transient loss of sleep results whenever brain concentrations of 5-HT are rapidly reduced, such as with raphe lesions, administration of PCPA in high doses, or withdrawal from high doses of 5-HTP; presumably, a new homeostatic balance develops after acute perturbation of brain 5-HT; (*b*) the loss of sleep that develops after raphe lesions or administration of PCPA may be reversed by administration of AMPT or chlorpromazine, agents that respectively block the synthesis of CA and DA receptors, thus antagonizing a hypothesized DA waking system which is balanced by 5-HT.

Whether or not 5-HT plays a role in "priming" REM sleep must also await further investigation. Such an interpretation is consistent with reports of decreased REM sleep following low doses of PCPA in man or methysergide and of increased REM following administration of low doses of 5-HTP or MAO inhibitors in man (8).

The importance of sleep factors can hardly be judged at the present time, if only because relatively little attention has been given to them. At this writing, no published accounts appear to exist on the effect of a sleep factor on the conventionally recorded sleep stages (REM and nonREM) sleep. Thus, it is not known whether these sleep factors produce normal physiological "sleep." The recent synthesis of delta sleep-inducing factor by Monnier et al (91), however, suggests that progress in this area may be rapid and exciting.

As in the case of 5-HT, the role of CA systems is not fully understood. Much evidence suggests that some DA and NE neurons may inhibit REM sleep, that is, acute studies that have either administered DA and NE agonists, α-receptor blockers (thymoxamine, phenoxybenzamine), or inhibitors of CA synthesis and storage (AMPT, reserpine), or that followed the activity of single "D-off" cells during REM sleep episodes. Conflicting evidence may come, however, from studies in which lesions of either the LC or mesencephalic tegmentum failed to affect total REM sleep time. Portions of the LC may be necessary, however, for the muscle atonia of REM sleep. Pharmacological blockade of DA receptors with chlorpromazine and similar agents in man and animals have not generally been shown to increase REM sleep. Some evidence also suggests that DA neurons are involved in cortical or behavioral activation; that is, acute administration of DA agonists increased arousal whereas lesions of DA neurons within ventral mesencephalic tegmentum reduced it. Again, however, pharmacologic blockade of DA receptors does not usually increase total

sleep time in man or animal, suggesting a distinction between central mechanisms for EEG and behavioral arousal.

Acute pharmacologic studies now indicate that cholinergic facilitation induces REM sleep and cortical activation, whereas cholinergic blockade inhibits REM sleep and promotes cortical deactivation. With chronic treatment, however, tolerance develops to the REM inhibitory effect of scopolamine. Furthermore, cholinergic mechanisms may be involved in the initiation of REM sleep rather than a maintenance or duration. Because of our limited knowledge of the neuroanatomic organization of the cholinergic neurons of the brain, a full interpretation of the role of the cholinergic system is premature.

At least two separate mechanisms have been described that can hasten the onset of REM sleep following the onset of nonREM sleep: cholinergic facilitation or α-adrenergic blockade. Sleep onset REM periods (wakefulness → REM → nonREM) appear, however, to be more difficult to induce experimentally by pharmacological techniques but have been reported with administration of physostigmine in reserpinized or brain stem–transected cats and of cholinergic agonists directly into brain stem in intact cats (51–56).

Sleep research in the future will undoubtedly benefit from many exciting new developments in neurobiology. Knowledge about the role of receptors has grown rapidly in recent years and a host of relatively unexplored transmitters and neuroregulators have been identified, whose role, if any, in sleep is relatively unknown (endorphin, enkephalin, substance P, neurotensin, somatostatin, adenosine, and others).

Literature Cited

1. Pieron, H. 1913. *Le Problème Physiologique du Sommeil.* Paris: Masson. 520 pp.
2. Jouvet, M. 1972. The role of monoamines and acetylcholine containing neurons in the regulation of the sleep-waking cycle. *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* 64:166–307
3. Hobson, J. A., McCarley, R. W., McKenna, T. M. 1976. Cellular evidence bearing on the pontine brain-stem hypothesis of desynchronized sleep control. *Prog. Neurobiol.* 6:280–376
4. Drucker-Colin, R. R., Spanis, C. W. 1976. Is there a sleep transmitter? *Prog. Neurobiol.* 6:1–22
5. Holman, R. B., Elliott, G. R., Barchas, J. 1975. Neuroregulators and sleep mechanisms. *Ann. Rev. Med.* 26:499–520
6. Mendelson, W. B., Gillin, J. C., Wyatt, R. J. 1977. *Human Sleep and Its Disorders.* Chaps. 2, 7. New York: Plenum. 260 pp.
7. Morgane, P. J., Stern, W. C. 1974. Chemical anatomy of brain circuits in relation to sleep and wakefulness. *Adv. Sleep Res.* 1:1–131
8. Wyatt, R. J. 1972. The serotonin-catecholamine dream bicycle: A clinical study. *Biol. Psychiatry* 5:33–63
9. Brodie, B. B., Shore, P. A. 1957. On a role for serotonin and norepinephrine as chemical mediators in the central autonomic nervous system. In *Hormones, Brain Function and Behaviour,* ed. H. Hoagland, pp. 161–76. New York: Academic
10. Brooks, D. C., Gershon, M. D. 1977. Amine repletion in the reserpinized cat: Effect upon PGO waves and REM sleep. *Electroencephalogr. Clin. Neurophysiol.* 42:35–47
11. Dement, W. C., Mitler, M. M., Henriksen, S. J. 1972. Sleep changes during chronic administration of parachlorophenylalanine. *Rev. Can. Biol.* 31: 239–46
12. Ursin, R. 1972. Differential effect of para-chlorophenylalanine on the two slow wave sleep stages in the cat. *Acta Physiol. Scand.* 86:278–85

13. Weitzman, E. D., Rappaport, M. M., McGregor, P., Jacoby, J. 1968. Sleep patterns of the monkey and brain serotonin concentrations: Effects of *p*-chlorophenylalanine. *Science* 160: 1361–63
14. Rechtschaffen, A., Lovell, R. A., Freedman, D. W., Whitehead, P. K., A drich, M. 1973. Effect of parachlorophenylalanine on sleep in the rat: Some implications for the serotonin-sleep hypothesis. In *Serotonin and Behavior,* ed. J. Barchas, E. Usdin, pp. 401–18. New York: Academic
15. Mendelson, W. B., Reichman, J., Othmer, E. 1975. Serotonin inhibition and sleep. *Biol. Psychiatry* 10:459–64
16. Tabushi, K., Himwich, H. E. 1971. Electroencephalographic study of the effects of methysergide on sleep in the rabbit. *Electroencephalogr. Clin. Neurophysiol.* 31:491–97
17. Adrien, J., Bourgoin, S., Hamon, M. 1977. Midbrain lesion in the newborn rat. I. Neurophysiological aspects of sleep. *Brain Res.* 127(1):99–110
18. Froment, J. L., Petitjean, F., Bertrand, N., Cointy, C., Jouvet, M., 1974. Effets de L'injection Intracérébrale de 5,6-Hydroxytryptamine sur les Monoamine Cérébrales et les Etats de Sommeil du Chat. *Brain Res.* 67:405–17
19. Bronzino, J. D., Morgane, P. J., Stern, W. C. 1972. EEG synchronization following application of serotonin to area postrema. *Am. J. Physiol.* 223:376–83
20. Hartmann, E. 1977. L-tryptophan as an hypnotic agent: A review. *Waking and Sleeping* 1:155–61
21. Fischer-Perroudon, C., Mouret, J., Jouvet, M. 1974. Sur un cas d'agrypnie (4 mois sans sommeil) au cours d'une maladie de Morvan. Effet favorable du 5-hydroxytryptophane. *Electroencephalogr. Clin. Neurophysiol.* 36:1–18
22. Guilleminault, C., Cathala, J. P., Castaigne, P. 1973. Effects of 5-hydroxytryptophan on sleep of a patient with a brain stem lesion. *Electroencephalogr. Clin. Neurophysiol.* 34:177–84
23. Aghajanian, G. K., Sheard, M. H. 1968. Behavioral effects of midbrain raphe stimulation—dependence on serotonin. *Commun. Behav. Biol.* Part A, 1:37–41
24. Jacobs, B. L., Asher, R., Dement, W. C. 1973. Electrophysiological and behavioral effects of electrical stimulation of the raphe nuclei in cats. *Physiol. Behav.* 11:489–95
25. Buckingham, R. L., Radulovacki, M. 1975. 5-hydroxyindoleacetic acid in cerebrospinal fluid: An indicator of slow wave sleep. *Brain Res.* 99:440–43
26. Wyatt, R. J., Neff, N. H., Vaughan, J., Franz, J., Ommaya, A. 1974. Ventricular fluid 5-hydroxyindoleacetic acid concentrations during human sleep. *Adv. Biochem. Psychopharmacol.* 11: 193–97
27. Sinha, A. K., Henriksen, S., Dement, W. C., Barchas, J. D. 1973. Cat brain amine content during sleep. *Am. J. Physiol.* 224:381–83
28. Kovacevic, R., Radulovacki, M. 1976. Monoamine changes in the brain of cats during slow-wave sleep. *Science* 193: 1025–27
29. McGinty, D. J., Harper, R. M. 1976. Dorsa raphe neurons: Depression of firing during sleep in cats. *Brain Res.* 101:569–75
30. Gillin, J. C., vanKammen, D. P., Graves, J., Murphy, D. L. 1975. Differential effects of d- and l-amphetamine on the sleep of depressed patients. *Life Sci.* 17:1233–40
31. Post, R. M., Gillin, J. C., Wyatt, R. J., Goodwin, F. K. 1974. The effect of orally administered cocaine on sleep of depressed patients. *Psychopharmacologia* 37:59–66
32. Haig, J. R., Schroeder, C. S., Schroeder, S. R. 1975. Effects of methylphenidate on hyperactive children's sleep. *Psychopharmacologia* 37:185–88
33. Autret, A., Minz, M., Beillevaire, T., Cathala, H. P., Castaigne, P. 1976. Suppression of paradoxical sleep by clonidine in man. *C. R. Acad. Sci.* 283: 955–57
34. Watson, R., Hartmann, E., Schildkraut, J. J. 1972. Amphetamine withdrawal: Affective state, sleep patterns, and MHPG excretion. *Am. J. Psychiatry* 129:263–69
35. Hartmann, E., Cravens, J. 1973. The effects of long term administration of psychotropic drugs on human sleep. II. The effects of reserpine. *Psychopharmacologia* 33:169–84
36. Coulter, J. D., Lester, B. K., Williams, H. L. 1971. Reserpine and sleep. *Psychopharmacologia* 19:134–47
37. Hartmann, E., Bridwell, T. J., Schildkraut, J. J. 1971. Alpha-methylparatyrosine and sleep in the rat. *Psychopharmacologia* 21:157–64
38. Oswald, I., Thacore, V. R., Adam, K., Brezinova, V., Burack, R. 1975. Alpha-adrenergic receptor blockage increases human REM sleep. *Br. J. Clin. Pharmacol.* 2:107–10

39. Hartmann, E., Zwilling, G. 1976. The effect of alpha and beta adrenergic receptor blockers on sleep in the rat. *Pharmacol. Biochem. Behav.* 5:135–38
40. Saga es, T., Erill, S. 1975. Effects of central dopaminergic blockage with pimozide upon the EEG stages of sleep in man. *Psychopharmacologia* 41:53–56
41. Saga es, T., Erill, S., Domino, E. F. 1969. Differentia effects of scopolamine and chlor romazine on REM and NREM sleep in normal male subjects. *Clin. Pharmacol. Ther.* 10:522–29
42. Henley, K., Morrison, A. R. 1974. A re-eva uation of the effects of lesions of the pontine tegmentum and locus coeruleus on ph nomena of paradoxica sleep in the cat. *Acta Neurobiol. Exp.* 34:215–32
43. Jones, B. E., Harper, S. T., Halaris, A. E. 1977. Effects of locus coeruleus lesions upon erebral monoamine ontent, sleep-wakefu ness states and the response to amphetamine in the cat. *Brain Res.* 124:473–96
44. Laguzzi, R., Petitj an, F., Pujol, J. F., Jouvet, M. 1971. Effets de l'injection intraventriculaire de 6-hydroxydopamine sur les états de sommeil et les monoamines cérébrales du chat. *C. R. Soc. Biol.* 165:1649–53
45. Jones, B. E., Bobi ler, P., Pin, C., Jouvet, M. 1973. The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking in the cat. *Brain Res.* 58:157–77
46. Chu, N. S., Bloom, F. E. 1974. Activity patterns of catecholamin -containing pontine neurons in the dorso-lateral tegmentum of unrestrained cats. *J. Neurobiol.* 5:527–44
47. Wyatt, R. J., Cantor, F., Gi lin, J. C., Gordon, E., Karoum, F., McCullough, D., Neff, N., Ommaya, A., Rauscher, F. P., Seaborg, J. B., Slaby, A. 1976. Ventricular fluid metabolites of phenolic amines and catecholamines. In *Trace Amines in the Brain,* ed. E. Usdin, M. Sand er, pp. 209–31. New York: Dekker
48. Mouret, J., Debil y, G., Renaud, B., Blois, R. 1976. Narcolepsy and hypersomnia: diseases or symptoms? Polygraphic and pharmacological studies. In *Narcolepsy,* ed. C. Guilleminault, W. C. Dement, P. Passouant, pp. 571–84. New York: Halsted
49. Renaud, B., Quincy, C., Mouret, J., Jouvet, M. 1975. La durée du sommeil paradoxal chez l'homme, relations eventuelles avec le metabolisme de la dopamine. *Nouv. Presse Med* 4:1656
50. Hartmann, E., Schildkraut, J. J. 1973. Desynchronized sleep and MHPG excretion: An inverse correlation. *Brain Res.* 61:412–16
51. Hernandez-Peon, R. 1965. A cholinergic hypnogenic limbic forebrain-hindbrain circuit. In *Neurophysiologie des Etats de Sommeil,* ed. M. Jouvet, pp. 63–88. Lyon: CNRS
52. George, R., Haslett, W. L., Jenden, D. J. 1964. A cho inergic mechanism in the brain stem reticular formation: Induction of parado ical sleep. *Int. J. Neuropharmacol.* 3:541–52
53. Baxter, B. L. 1969. Induction of both emotional behavior and a novel form of REM sleep by chemical stimulation app ied to cat mesencephalon. *Exp. Neurol.* 23:220–30
54. Amatruda, T. T., Black, D. A., McKenna, T. M., McCarley, R. W., Hobson, J. A. 1975. Sleep cycle control and cho inergic mechanisms: Differential effects of carbachol of pontine brain stem sites. *Brain Res.* 98:501–15
55. Mitler, M. M., Dement, W. C. 1974. Cataplectic- ike behavior in cats after microinjection of carbachol in pontine reticular formation. *Brain Res.* 68: 335–43
56. Karczmar, A. G., Longo, V. G., S ott de Carolis, A. 1970. A pharmacological model of paradoxical sleep: The role of cholinergic and monoamine systems. *Physiol. Behav.* 5:175–82
57. Sitaram, N., Wyatt, R. J., Dawson, S., Gillin, J. C. 1976. REM sleep induction by physostigmine infusion during sleep in normal volunteers. *Science* 191: 1281–83
58. Sitaram, N., Mendelson, W. B., Wyatt, R. J., Gillin, J. C. 1977. The time-dependent induction of REM sleep and arousa by physostigmine infusion during normal human sleep. *Brain Res.* 122:562–67
59. Domino, E. F., Yamamoto, K., Dren, A. T. 1968. Role of cho inergic mechanisms in states of wakefulness and sleep. In *Anticholinergic Drugs and Brain Function in Animals and Man, Prog. Brain Res.,* ed. P. B. Bradley, M. Fink, Vol. 28. Amsterdam: E sevier
60. Hazra, J. 1970. Effect of hemicholinium-3 on slow wave and paradoxical sleep of cat. *Eur. J. Pharmacol.* 11:395–97
61. Toyada, J., Sasaki, K., Kurihara, M. 1966. A polygraphic study on the effect

of atropine on human nocturnal sleep. *Folia Psychiatr. Neurol. Jpn.* 20:275–89
62. Weiss, T., Bohjanecky, Z., Fifkova, E., Roldan, E. 1964. Influence of atopine on sleep cycle in rats. *Psychopharmacologia* 5:126–35
63. Pompeiano, O. 1972. Reticular control of the vestibular nuclei: Physiology and pharmacology. *Prog. Brain Res.* 37: 614–18
64. Vimont-Vicary, P. 1965. La suppression des differents états de sommeil. Etudo compartementals, EEG, et neuropharmacologique chez le chat. Thése Univ. Lyon. 95 pp.
65. Jacobs, B. L., Henriksen, S. J., Dement, W. C. 1972. Neurological bases of the PGO waves. *Brain Res.* 48:406–11
66. Ruch-Monachan, M. A., Jalfie, M., Haefely, W. 1976. Drugs and PGO waves in the lateral geniculate body of the curarized cat. *Arch. Int. Pharmacodyn. Ther.* 219:251–346
67. Magherini, P. C., Pompeiano, O., Thoden, U. 1971. The neurochemical basis of REM sleep: A cholinergic mechanism responsible for rhythmic activation of the vestibulo-oculomotor system. *Brain Res.* 35:565–69
68. Celesia, G. G., Jasper, H. H. 1966. Acetylcholine released from cerebral cortex in relation to state of activation. *Neurology* 16:1053–64
69. Gadea-Ciria, M., Stadler, H., Lloyd, K. G., Bartholini, G. 1973. Acetylcholine release within the cat striatum during the sleep-wakefulness cycle. *Nature* 243:518–19
70. Haranath, P. S. R. K., Venkatakrishna-Bhatt, H. 1973. Release of acetylcholine from perfused cerebral ventricles in unanaesthetized dogs during waking and sleep. *Jpn. J. Physiol.* 23:241–50
71. Hoshino, K., Pompeiano, O., Magherini, P. C., Mergner, T. 1976. Oscillatory activity of pontine neurons related to the regular occurrence of REM bursts in the decerebrate cat. *Brain Res.* 116:125–30
72. Siegel, J. M., McGinty, D. J. 1977. Pontine reticular formation neurons: Relationship of discharge to motor activity. *Science* 196:678–80
73. Papp, M., Bozsik, G. 1966. Comparison of the cholinesterase activity in the reticular formation of the lower brain stem of cat and rabbit. *J. Neurochem.* 13:697–703
74. Bradley, P. B., Dray, A. 1972. Short-latency excitation of brain stem neurons in the rat by acetylcholine. *Br. J. Pharmacol.* 45:584–95
75. Richter, D., Crossland, J. 1949. Variation in acetylcholine content of the brain with physiological state. *Am. J. Physiol.* 159:247–55
76. Stepita-Klavco, M., Dolezalova, H., Fairweather, R. 1974. Piperidine increase in the brain of dormant mice. *Science* 183:536–37
77. Dolezalova, H., Stepita-Klavco, M., Fairweather, R. 1974. The accumulation of piperidine in the central ganglia of dormant snails. *Brain Res.* 72:115–22
78. Bowers, M. B. Jr., Hartmann, E. L., Freedman, D. 1966. Sleep deprivation and brain acetylcholine. *Science* 153:1416–17
79. Tsuchiya, K., Toru, M., Kobayashi, T. 1969. Sleep deprivation: Changes of monoamines and acetylcholine in rat brain. *Life Sci.* 8(1):867–73
80. Ghosh, P. K., Hrdina, P. D., Ling, G. M. 1975. Effects of REMS deprivation on striatal dopamine and acetylcholine in rats. *Pharmacol. Biochem. Behav.* 4:401–5
81. Sagales, T., Domino, E. F. 1973. Effects of stress and REM sleep deprivation on the patterns of avoidance learning and brain acetylcholine in the mouse. *Psychopharmacologia* 29(4):307–15
82. Safer, D. J., Allen, R. P. 1971. The central effects of scopolamine in man. *Biol. Psychiatry* 3:347–55
83. Schnedorf, J. G., Ivy, A. C. 1938. An examination of the hypnotoxin theory of sleep. *Am. J. Physiol.* 125:491–505
84. Pappenheimer, J. R., Koski, G., Fencl, V., Karnovsky, M. L., Krueger, J. 1975. Extraction of sleep-promoting Factor S from cerebrospinal fluid and from brains of sleep-deprived animals. *J. Neurophysiol.* 38:1299–1311
85. Pappenheimer, J. R., Fencl, V., Karnovsky, M. L., Koski, G. 1974. Peptides in cerebrospinal fluid and their relation to sleep and activity. *Res. Publ. Assoc. Res. Nerv. Ment. Dis.* 53:201–8
86. Sachs, J., Ungar, J., Waser, P. G., Borbeley, A. A. 1976. Factors in cerebrospinal fluid affecting motor activity in the rat. *Neurosci. Lett.* 2:83–86
87. Ringle, D. A., Herndon, B. L. 1969. Effects on rats of CSF from sleep deprived rabbits. *Pflügers Arch.* 306: 320–28
88. Nagasaki, H., Iriki, M., Inoue, S., Uchizono, K. 1974. The presence of sleep-promoting material in the brain of

sleep deprived rats. *Proc. Jpn. Acad.* 50:241–46
89. Nagasaki, H., Iriki, M., Uchizono, K. 1976. Inhibitor effect of the brain extract from sleep-deprived rats (BE-SDR) on the spontaneous discharges of crayfish abdominal ganglion. *Brain Res.* 109:202–5
90. Monnier, M., Dudler, L., Schoenenberger, G. A. 1973. Humoral transmission of sleep. VIII. Effects of the "Sleep Factor Delta" on cerebral motor, and visceral activities. *Pflügers Arch.* 345: 23–35
91. Monnier, M., Dudler, L., Gaechter, R., Maier, P. F., Tobler, H. J., Schoenenberger, G. A. 1977. The delta sleep inducing peptide (DSIP). Comparative properties of the original and synthetic nonapeptide. *Experientia* 33(4):548–52
92. Matsumoro, J., Sogare, K., Hori-Santiago, Y. 1972. Sleep in parabiosis. *Experientia* 28:1043–44
93. Lenard, H. G., Schulte, F. J. 1972. Polygraphic sleep study in craniopagus twins. *J. Neurol. Neurosurg. Psychiatry* 35:756–60
94. DeAndres, I., Gutierrez-Rivas, E., Nava, E., Reinoso-Suarez, F. 1976. Independence of sleep-wakefulness cycle in an implanted head "encephale-isole." *Neurosci. Lett.* 2:13–18