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C. L. SPINWEBER
R. URSIN
R. P. HILBERT
R. L. HILDERBRAND

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NAVAL HEALTH RESEARCH CENTER
P. O. BOX 85122
SAN DIEGO, CALIFORNIA 92138

NAVAL MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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L-TRYPTOPHAN: EFFECTS ON DAYTIME SLEEP LATENCY AND THE WAKING EEG

Cheryl L. Steinweber, Reidun Ursin,[1] Raymond P. Hilbert and Richard L. Hilderbrand[2]

Clinical Psychophysiology Department

Naval Health Research Center

P.O. Box 85122

San Diego, CA 92138-9174

[1]Visiting Scientist, on leave from the Institute of Physiology, University of Bergen, Norway, at the time this research was conducted.

[2]Now at the Naval Biosciences Laboratory, Naval Supply Center, Oakland, CA.

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SUMMARY

The essential amino acid, l-tryptophan, has been shown to reduce sleep latency when administered in doses ranging from 1–15 grams. Because l-tryptophan is regularly included in dietary protein foods, it has been called a "natural hypnotic." At least one author has suggested that plasma tryptophan, which shows a diurnal rhythm with peak levels in the late evening hours, may be a physiological regulator of sleep onset.

However, not all researchers have found that l-tryptophan reduces sleep latency. There is also controversy about whether l-tryptophan administration alters the pattern of recorded sleep. Finally, the underlying mechanism for the putative hypnotic effects has not been well-established, although serotonergic systems are most likely involved.

We conducted this study to determine the effects of l-tryptophan (4 g) on the waking EEG and on daytime sleep. Twenty normal, drug-free adults participated. Subjects were assigned to a morning or afternoon group, and data were collected on two occasions, after l-tryptophan and after placebo, assigned in a counter-balanced order. Blood samples were obtained by venepuncture and later analyzed for total and free tryptophan levels. Daytime nap sleep was recorded and scored according to usual procedures. Waking EEGs were digitized on-line and later analyzed for changes in five frequency bands: 16–40 Hz (beta), 13.0–15.5 Hz (sigma), 8.0–12.5 Hz (alpha), 4.0–7.5 Hz (theta), and 0.5–3.5 Hz (delta).

L-tryptophan significantly reduced sleep latency without altering nap sleep stages and elevated plasma total and free tryptophan levels. During waking EEGs, l-tryptophan significantly increased alpha time, theta time, and theta intensity and significantly decreased alpha frequency. No wave bands were altered during sleep.

Our results indicate that l-tryptophan is an effective daytime hypnotic which facilitates sleep onset at clock times which do not coincide with biological sleep times. The changes observed in the waking EEG suggest that l-tryptophan may act by lowering arousal level during the awake state, thus setting the stage for more rapid sleep onset. These results are of importance in that they demonstrate the sleep-facilitating role of l-tryptophan in subjects who do not usually nap at the scheduled nap times used in this study. The implication is that l-tryptophan is adequately deactivating to facilitate sleep onset at clock times other than normal bedtimes, particularly when the environment is conducive to sleep. Thus, l-tryptophan may be useful in alleviating sleep onset difficulties produced by time zone changes and fragmented sleep schedules as well as in the clinical management of some nighttime sleep-onset insomniacs

INTRODUCTION

The hypnotic efficacy of the amino acid l-tryptophan has been the subject of substantial research interest. The inconsistencies in reported findings have engendered much scientific debate. Two areas of controversy include the efficacy of l-tryptophan in sleep induction and maintenance, and its effects on sleep measures.

In various studies using a wide range of subject-types, sleep latency has been reported to be significantly reduced by l-tryptophan in doses ranging from 1—15 g (Hartmann, 1967; Williams, Lester, & Coulter, 1969; Griffiths, Lester, Coulter, & Williams, 1972; Hartmann, Cravens, & List, 1974; Hartmann & Elion, 1977; Brown, Horrom, & Wagman, 1979; Hartmann & Spinweber, 1979). Other investigators, however, did not report sleep-facilitating effects (Wyatt, Engelman, Kupfer, Fram, Sjoerdsma, & Snyder, 1970; Březinová, Loudon, & Oswald, 1972; Adam & Oswald, 1979; Nicholson & Stone, 1979; Small, Milstein, & Golay, 1979). Decreased time awake and/or increased total sleep time have been found in some laboratory studies (Williams *et al.*, 1969; Wyatt *et al.*, 1970; Griffiths *et al.*, 1972; Hartmann *et al.*, 1974). In subjects required to remain awake, early evening administration of l-tryptophan (2 and 4 g doses) has been demonstrated to increase subjective ratings of sleepiness on the Stanford Sleepiness Scale (Hartmann, Spinweber, & Ware, 1976; Spinweber, 1977).

Discrepancies in the reported effects of l-tryptophan on sleep measures may be due, in part, to the various dose levels employed. Hartmann *et al.* (1974) reported that low doses of l-tryptophan (1—5 g) did not produce alterations in sleep measures. A review of data from a number of low dose studies conducted by Hartmann and his colleagues indicates a general trend toward increased slow wave sleep (SWS), although this effect in the individual studies does not reach significance (Hartmann & Spinweber, 1979). At larger doses (10—15 g), Hartmann *et al.* (1974) found reductions in rapid eye movement (REM) sleep and increases in SWS. Other investigators, using doses ranging from 5—12 g, have also reported tryptophan-induced changes in EEG sleep, but the types of reported changes are not consistent, particularly regarding effects on REM latency (Oswald, Ashcroft, Berger, Eccleston, Evans, & Thacore, 1966; Wyatt *et al.*, 1970) and amount of REM sleep (Williams *et al.*, 1969; Wyatt *et al.*, 1970; Griffiths *et al.*, 1972). There is agreement that a 7.5 g dose increases SWS (Williams *et al.*, 1969; Wyatt *et al.*, 1970; Griffiths *et al.*, 1972).

The mechanism underlying the hypnotic effects of l-tryptophan has not been established. It has been posited that the effects are due to the conversion of l-tryptophan to serotonin in the brain (Oswald *et al.*, 1966; Hartmann, Chung, & Chien, 1971). Serotonin levels in brain are determined by the availability of l-tryptophan in plasma, and plasma levels are responsive to the tryptophan content of dietary protein (Moir & Eccleston, 1968; Young, Hussein, & Murray, 1969; Fernstrom & Jacoby, 1975; Wurtman & Fernstrom, 1976). Serotonergic mechanisms have been implicated in the control of SWS (Jouvet, 1972) and, more recently, in the modulation of the waking state. Serotonin precursors administered to cats deactivate the waking state without altering sleep measures (Ursin, 1976). Unit activity of cat dorsal raphe nucleus, containing primarily serotonergic neurons, is highest in the waking state and progressively decreases as sleep onset time nears, indicating modulation of activation levels by these neurons (Trulson & Jacobs, 1979). Raphe lesions in rats have only short-lasting effects on sleep while the effects on activity during waking are prolonged (Mouret & Coindet, 1980). Thus, recent animal data suggest that l-tryptophan, acting via a serotonergic mechanism, may modify the waking state rather than potentiating sleep mechanisms *per se*. The inconsistent findings on sleep stage effects further suggest that non-sleep mechanisms may be influenced by l-tryptophan administration.

Many metabolic factors may act to alter the availability of l-tryptophan to the brain at different times in the diurnal cycle (Knox, 1966; Knox, Piras, & Tokuyama, 1966; Azmitia & McEwen, 1969; Hardeland, 1969; Fernstrom & Wurtman, 1972; Tagliamonte, Biggio, Vargiu, & Gessa, 1973; Curzon & Knott, 1974; Gessa & Tagliamonte, 1974), and it has been previously suggested that l-tryptophan may have hypnotic effects only in nighttime administration (Cooper, 1979). We conducted this study of l-tryptophan to determine its effects on the waking and sleep EEG and to assess its hypnotic efficacy in daytime administration. Although previous reports indicate that 1 g of l-tryptophan is the smallest dose having hypnotic effects in bedtime administration (Hartmann & Spinweber, 1979), studies on subjective sleepiness have shown that, in the earlier evening hours, 4 g produce increases in subjective sleepiness (Hartmann *et al.*, 1976) while a 1 g dose is ineffective (Spinweber & Hartmann, unpublished data). Based on such data, we chose the 4 g dose for daytime study.

METHOD

Subjects

Subjects were 15 male and 5 female employees of the Naval Health Research Center, San Diego (mean age 32.8 ± 8.7 years, range 20–53 years). All subjects were drug-free and reported no sleep, psychological, or medical problems.

Procedure

Subjects were assigned to either a morning (N = 10) or afternoon (N = 10) nap group. Each subject took two daytime naps, scheduled exactly one week apart. On each occasion, the subject received tablets of l-tryptophan (4 g) or matching placebo tablets, assigned in a counter-balanced order. Both subjects and researchers were blind to treatment conditions. Female subjects were not scheduled for participation during the premenstrual or menstrual week because of previously demonstrated alterations in sleep requirement and sleep pattern at the time of menses (Hartmann, 1966). Subjects were instructed to finish breakfast prior to 0700 (morning group) or lunch prior to 1100 (afternoon group) on study days and requested to maintain similar diets on the two occasions. They were further instructed not to consume alcohol or any medications on study days and to maintain their regular sleep schedule throughout the study week. Subjects kept dietary records for the 24-hour period prior to each study session and at-home sleep logs for the study week.

The procedure for the morning (AM) and afternoon (PM) nap sessions was identical (see Table 1). Subjects completed the Thayer Adjective Checklist (Thayer, 1967, 1978) and Stanford Sleepiness Scale (SSS) (Hoddes, Zarcone, Smythe, Phillips, & Dement, 1973) prior to drug administration, again 25 minutes post-drug administration, and after the nap. Blood samples were drawn 30 minutes after drug administration. Subjects were instructed to stay awake during the waking EEG recordings, and the lights were on during all waking recordings. At 1 hour post-drug, subjects were instructed to take a nap and the lights were turned off.

Sleep Data

Seven channels of data were polygraphically recorded: three EEG channels (F_3, C_3, and O_1, each referred to linked mastoid reference $A_1 + A_2$); two EOG channels, the outer canthus of each eye referred to the linked reference (E_1 versus $A_1 + A_2$, E_2 versus $A_1 + A_2$), one EMG channel (bipolar submental), and one EKG channel. Nap records were scored in 30-second epochs for sleep stages according to the standardized procedures (Rechtschaffen & Kales, 1968) by one of two experienced scorers blind to treatment conditions; a check on page-by-page independent scoring showed a 97% agreement between the two scorers. The waking EEGs were scored for the presence of waking and Stage 1 using 10-second epochs.

TABLE 1

Schedule for L-tryptophan Study

Procedure	Morning Group	Afternoon Group
Subject arrives: apply electrodes	0830	1230
Pre-drug SSS and Thayer	0845	1245
Pill administration (l-tryptophan, 4 g, or placebo)	0850	1250
Post-drug SSS and Thayer	0915	1315
Blood sample drawn	0920	1320
Waking EEG, eyes open	0930	1330
Waking EEG, eyes closed	0940	1340
Nap period, lights out	0950	1350
Waking EEG, eyes open	1150	1550
Waking EEG, eyes closed	1155	1555
Post-nap SSS and Thayer, Post-nap questionnaire	1200	1600
Session completed: remove electrodes	1205	1605

EEG Wave Band Analysis

Two EEG channels (C_3-A_1+A_2, O_1-A_1+A_2) were digitized on-line at a rate of 128 samples/second with resolution of twelve bits. The data were then stored on magnetic tape for later analysis.

The EEG for each recording category (pre-nap eyes open and closed, post-nap eyes open and closed) in each treatment condition (placebo or l-tryptophan) was analyzed for changes in five EEG frequency bands. To be sure that results based on analyses of 10-minute and 5-minute total time samples were not influenced by the presence of artifacts, we also selected artifact-free 1-minute EEG samples on which to perform the same analyses. These 1-minute samples were comprised of three 20-second periods or, occasionally, two 30-second periods selected from different parts of each awake record. Selection of 1-minute samples was done blind with regard to treatment condition.

Artifact-free 1-minute samples were also selected from the various sleep stages according to preestablished criteria. Samples were selected as close to 2 hours after the medication was given as possible. For Stage 2, the first and last minutes of a period were avoided. Samples from REM sleep were selected during periods with rapid eye movements, if possible. Not all subjects showed all sleep stages in daytime naps.

The following EEG frequency bands were analyzed: 16—40 Hz (beta), 13.0—15.5 Hz (sigma, obtained only from the 1-minute samples), 8.0—12.5 Hz (alpha), 4.0—7.5 Hz (theta), and 0.5—3.5 Hz (delta). The occipital EEG was used for analysis of alpha activity. All other wave band analyses were performed on the central EEG. Intensity in $\mu V^2/(c/sec)$ for each frequency band was obtained by Fourier analysis (Fast Fourier Transform) on 2-second data epochs, with frequency resolution of 0.5 Hz. Time present (seconds/minutes) of alpha and theta was determined by peak-to-peak analysis. For this analysis, the alpha band was defined as 8.0—12.8 Hz. The mean frequency for the alpha and theta bands was also obtained.

Blood Sample Data

Blood samples were collected by venepuncture into 7.0 ml evacuated tubes containing 9 mg of $(Na)_2EDTA$ and mixed gently. Following centrifugation at 1500 X g for 5 minutes, the blood plasma was

removed by Pasteur pipette. A 1 ml aliquot was placed in a polyethylene vial and frozen immediately. A 1 ml aliquot was placed in a 13 mm stirred cell (Millipore Corporation) for filtration through a Pillicon filter (#PTGC 01310, Millipore Corporation) which retains materials with a molecular weight greater than approximately 10^4 daltons. Filtration was performed at two atmospheres pressure under 2.5% CO_2 in air. The gas mixture was introduced through a 3-way valve and pressure was maintained by a weight on a syringe attached to the top of the stirred cell. The initial few drops of filtrate were discarded, and filtrate was then collected, placed in a polyethylene vial, and frozen.

Amino acid analyses were performed on plasma and plasma filtrate samples using a Durrum automated amino acid analyzer. Sulfosalicylic acid (10 mg/200 µl plasma) was used to precipitate protein, and 50 µl of plasma or plasma filtrate deproteinate was applied to the column for analysis. The run was continued for a sufficient period to allow the elution of tryptophan (approximately 114 minutes). Basic amino acids were not determined. Results were reported as picomoles/50 ml.

Statistical Analysis

The effect of l-tryptophan on sleep latency, a non-normally distributed sleep measure, was statistically analyzed using the Wilcoxon Matched Pairs Signed Ranks Test. Sleep latency was defined as the time in minutes from lights out to the onset of the first Stage 2 or, for one subject in the morning group, to the onset of REM sleep. Sleep onset REM periods, while characteristic of narcoleptic sleep, also have been reported to occur in morning naps of normal sleepers (Globus, 1966; Webb, Agnew, & Sternthal, 1966; Kelley, Laughlin, Carpenter, Simmons, Sidoric, & Lentz, 1973; Weitzman, Nogeire, Perlow, Fukushima, Sassin, McGregor, Gallagher, & Hellman, 1974; Carskadon & Dement, 1975; Moses, Hord, Lubin, Johnson, & Naitoh, 1975). Comparisons between the placebo and l-tryptophan conditions for sleep, plasma, and subjective measures were performed using *t*-tests for correlated means (df = 19). Significant comparisons were verified using the Wilcoxon Matched Pairs Signed Ranks Test. The possibility that l-tryptophan might have differential effects on sleep latency according to time of day (AM versus PM group) was tested by performing an independent *t*-test (df = 18) on the difference scores (placebo minus tryptophan sleep latency) for the two groups.

Analysis of the waking EEG measures was performed using program 2V (Analysis of Variance, ANOVA, for Repeated Measures) of the BMDP statistical package. The omnibus ANOVA included one grouping factor, nap group (AM or PM), and three trial factors, treatment condition (placebo or tryptophan), time (pre- or post-nap), and the eyes condition (open or closed). Because of procedural error, the post-nap eyes-closed EEG recording in the l-tryptophan condition was not obtained from one PM group subject. This subject's data were not included in the omnibus *F* tests performed on the waking EEG data. For analysis of EEG samples from the nap period, ANOVA factors were nap group (AM or PM) and treatment condition (placebo or tryptophan). There was no significant main effect of the factor nap group in any ANOVA performed. Post-hoc *t*-tests for correlated means (df = 19) were used to identify the sources of significant effects. All *t*-tests were two-tailed.

RESULTS

Effects on Sleep Latency

All subjects showed sleep onset during the nap period under both treatment conditions. L-tryptophan significantly reduced sleep latency from 23.6 ± 23.9 minutes on placebo to 12.6 ± 9.8 minutes (T(Wilcoxon) = 49, $P < 0.05$). There was no differential effect of time of day (AM versus PM group) on the reduction in sleep latency. Latency to the first Stage 1 was also significantly reduced from 12.6 ± 19.0 minutes on placebo to 4.9 ± 4.6 minutes on l-tryptophan (T(Wilcoxon) = 41, $P < 0.02$).

TABLE 2

Sleep Measures for Daytime Naps (minutes)

Sleep Measure	Placebo	L-tryptophan
	$\bar{X}$ ± (S.D.)	$\bar{X}$ ± (S.D.)
Sleep latency	23.40 (23.90)	12.60 (9.80)*
Total bed time	119.80 (0.34)	119.80 (0.38)
Total sleep (Stages 2, 3, 4, REM)	66.78 (26.99)	74.95 (31.08)
Stage 1	14.05 (9.62)	16.38 (7.78)
Stage 2	43.35 (16.53)	53.75 (19.76)
Stage 3	9.57 (8.57)	6.30 (7.38)
Stage 4	4.70 (8.25)	4.63 (8.36)
SWS (Stages 3 + 4)	14.27 (12.20)	10.92 (13.69)
REM	9.15 (12.00)	10.27 (11.20)
Total Stage Wake	38.65 (28.52)	27.95 (29.14)

*$P < 0.05$, Wilcoxon Matched Pairs Signed Ranks Test

Effects on Daytime Sleep

The results of comparison of sleep stages for the placebo nap and l-tryptophan nap are presented in Table 2. No comparison was significant for both the normal-based and distribution-free tests. Administration of l-tryptophan did not alter any EEG wave bands during sleep Stages 2, 3, 4 or REM.

Effects on the Waking EEG

(A) Appearance of Stage 1 during Waking EEGs

Stage 1 EEG often appeared during the eyes-closed recordings in both the placebo and l-tryptophan conditions. The latency to the first 10-second epoch of Stage 1 was significantly shorter after l-tryptophan ($\bar{X}$ = 138.5 ± 37.4 seconds) than after placebo ($\bar{X}$ = 249.5 ± 48.3 seconds) (T(Wilcoxon) = 25, $P < 0.01$). When questioned after having presented 10 seconds Stage 1 EEG, most subjects denied having been asleep.

(B) Wave Band Analyses

L-tryptophan administration altered alpha and theta activity in awake subjects but had no effect on beta, delta, or sigma intensity. Results obtained from analyses of total time samples and 1-minute samples of waking EEGs were highly consistent. Therefore, the results from total time samples are described below, while results from 1-minute samples are presented in Figs. 1 and 2.

(1) Alpha Activity. Alpha time was significantly increased by l-tryptophan administration ($F_{1,17} = 8.21$, $P < 0.02$). This effect was most strong during the eyes-open recording conditions, both in the pre-nap ($t_{19} = 2.489$, $P < 0.05$) and post-nap ($t_{19} = 3.082$, $P < 0.01$) EEGs. Because of high individual variability in alpha intensity measured as $\mu V^2/(c/sec)$, the omnibus ANOVA for this measure did not yield significant results for the total time samples. However, in the pre-nap eyes-open condition, 15 of 20 subjects showed increased alpha intensity following l-tryptophan administration ($P < 0.05$, Sign test). Mean frequency of alpha was significantly slowed by l-tryptophan ($F_{1,17} = 10.21$, $P < 0.005$). Post-hoc t-tests showed that alpha frequency was reduced during the post-nap eyes-open condition ($t_{19} = 2.542$, $P < 0.05$). Analyses of samples of awake EEGs recorded during the 2-hour nap session gave

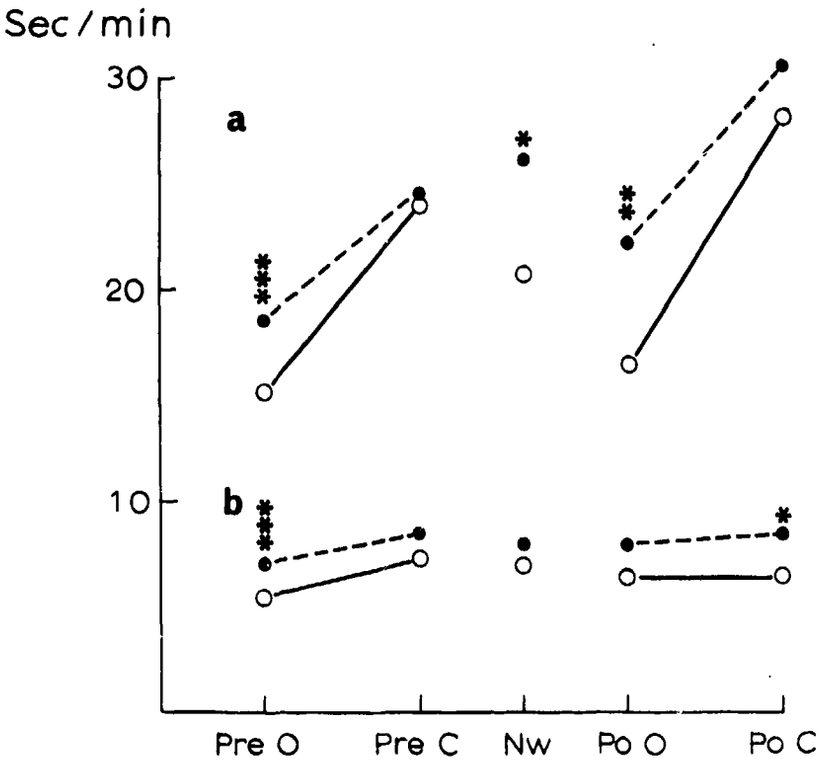

Fig. 1. Time present of alpha (a) and theta (b) activity in 1-minute samples from the waking EEGs and nap time Stage Wake. Placebo: open circles, solid line. L-tryptophan: dots, broken line. Pre O: pre-nap eyes open. Pre C: pre-nap eyes closed. Nw: nap time Stage Wake. Po O: post-nap eyes open. Po C: post-nap eyes closed. Asterisks indicate significance level of post-hoc t-tests, ***$P < 0.01$, **$P < 0.02$, and *$P < 0.05$, respectively.

consistent results: increased alpha time ($F_{1,18} = 5.66$, $P < 0.03$) and alpha intensity ($F_{1,18} = 10.36$, $P < 0.005$) on l-tryptophan.

(2) Theta Activity. L-tryptophan produced an overall increase in theta time ($F_{1,17} = 10.88$, $P < 0.004$). Theta time was significantly increased compared to placebo values during the pre-nap eyes-open ($t_{19} = 2.755$, $P < 0.02$), pre-nap eyes-closed ($t_{19} = 2.576$, $P < 0.02$), and post-nap eyes-closed ($t_{18} = 2.568$, $P < 0.02$) EEG recordings. Theta intensity was significantly increased by l-tryptophan ($F_{1,17} = 7.50$, $P < 0.02$). Post-hoc t-tests on theta intensity reached significance for the pre-nap eyes-open ($t_{19} = 2.498$, $P < 0.05$), pre-nap eyes-closed ($t_{19} = 3.471$, $P < 0.01$), and post-nap eyes-open ($t_{19} = 2.115$, $P < 0.05$) conditions. In analyses of nap period 1-minute samples, theta intensity was signif'antly increased during Stage Wake ($F_{1,18} = 9.35$, $P < 0.01$), and theta time was increased during Stage 1 ($F_{1,18} = 5.31$, $P < 0.04$).

Effects on Plasma L-tryptophan Levels

Administration of l-tryptophan significantly elevated plasma total tryptophan levels 313 ± 260% above placebc levels ($t_{19} = 6.11$, $P < 0.001$) and free tryptophan levels 372 ± 343% above placebo levels ($t_{19} = 5.72$, $P < 0.001$).

Subjective Measures and Side Effects

Self-reported sleepiness (SSS) and deactivation (Thayer) were not increased when assessed at 25 minutes post-drug or at the close of the study session. Subjects were questioned after the study

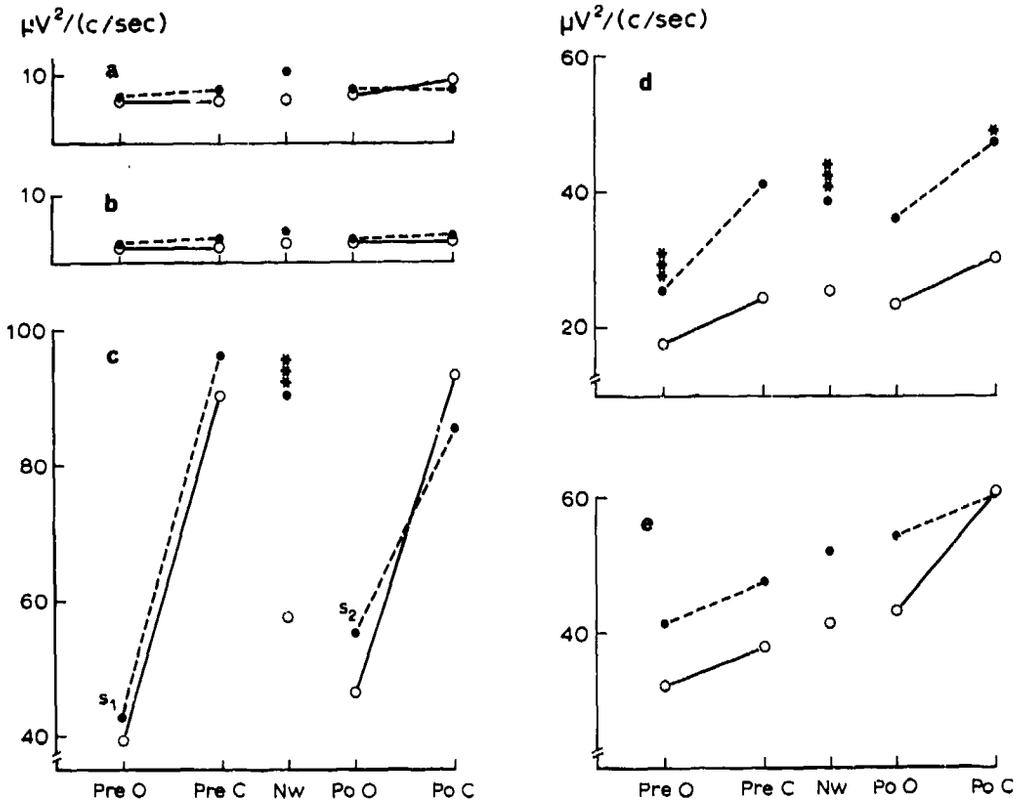

Fig. 2. Intensity of beta (a), sigma (b), alpha (c), theta (d), and delta (e) from 1-minute samples of waking EEGs and nap time Stage Wake. Significant Sign tests, S_1 $P < 0.001$ and S_2 $P < 0.05$. Other symbols and abbreviations as in Fig. 1.

session and during the following week about possible side effects. In general, reports of post-nap "grogginess," "tiredness," and "nausea" were few and distributed evenly between the two conditions. However, one case of severe nausea during the nap session was associated with the l-tryptophan condition.

DISCUSSION

Our study demonstrated that 4 g l-tryptophan significantly reduces daytime sleep latency without alteration of sleep stages. The EEG frequency analyses suggest that l-tryptophan modifies the awake state. Increased alpha and theta time, increased theta intensity, and decreased alpha frequency were found in the waking recordings, particularly in the eyes-open conditions. Together, these EEG changes indicate deactivation and lower arousal level (O'Hanlon & Beatty, 1977) during the awake state. Previously, Greenwood, Lader, Kantameneni, & Curzon (1975) reported an increase in the voltage of the 4.0–7.5 Hz and 2.4–4.0 Hz frequency bands after tryptophan loading. While these authors did not report any change in the alpha band, they recorded the EEG during performance of a reaction time task, an experimental situation which may have precluded effects on alpha activity. In awake cats, l-tryptophan loading increased a specific form of synchronous EEG activity (Ursin, 1976) which has been compared to human alpha activity (Rougeul, Corvisier, & Letalle, 1974). This same activity was also increased by administration of the immediate serotonin precursor, 5-hydroxytryptophan, indicating that the effect was serotonergic (Ursin, 1976). In consideration of these EEG findings, the reduction of sleep latency by l-tryptophan may be interpreted as a consequence of deactivation of the waking state. The effects are most likely mediated via brain serotonergic neurons. Our findings and interpretation are consistent with recent models of serotonergic functions in waking and sleep, which stress the

deactivating or activation-modulating effects of serotonergic neurons (Ursin, 1976; Trulson & Jacobs, 1979; Mouret & Coindet, 1980). These models suggest that any sleep changes which occur when serotonergic transmission is potentiated or blocked are secondary to effects on waking (Jacobs & Jones, 1977). The fact that we and others (Hartmann *et al.*, 1974) did not find effects of l-tryptophan on sleep stages *per se* is consistent with this hypothesis.

Our data indicate that l-tryptophan administration alters plasma levels relatively quickly, within 30 minutes of ingestion. However, hypnotic effects, both subjectively and objectively measured, occur later, presumably, in our model, due to the time required for uptake of l-tryptophan into the brain and potentiation of serotonergic systems. In the present study, subjective measures of sleepiness were not significantly elevated when measured 25 minutes following l-tryptophan administration. A review of previous studies on subjective sleepiness (Hartmann *et al.*, 1976; Spinweber, 1977, 1981) suggests that significant subjective effects are not evident until approximately 45 minutes after administration, although the appropriate trend is present in the data 15—30 minutes post-ingestion.

Demonstration of effects of l-tryptophan on EEG-recorded sleep latency also appears to depend upon the time post-administration at which "lights out" occurs. For example, our conclusion that l-tryptophan is effective as a daytime hypnotic is not consistent with results of a daytime study by Nicholson & Stone (1979) who found no sleep-facilitating effects. In their study, l-tryptophan (4 g) or placebo was administered at 1400, and this time point was also chosen as "lights out" for measurement of sleep latencies. In the placebo condition, the mean latency from lights out to Stage 2 was 22.2 minutes and, on l-tryptophan (4 g), was 21.8 minutes. We suggest that the subjects in the Nicholson & Stone study fell asleep too quickly after pill administration and lights out to permit identification of a sleep-facilitating effect. When considering all available data, it appears that increases in subjective sleepiness, deactivation of the waking EEG, and concomitant reduction of sleep latency become significant effects between 40 minutes to 1.25 hours post-ingestion of l-tryptophan. This conclusion is consistent with pharmacokinetic data provided by Domino & Krause (1974), who demonstrated that plasma total tryptophan levels peak at 1.2 ± 0.2 hours post-ingestion of a 32 mg/kg loading dose, administered to normal human subjects who remained awake.

It has been previously suggested that l-tryptophan is a "natural" hypnotic (Wyatt *et al.*, 1970; Hartmann *et al.*, 1971). The results of our study indicate that l-tryptophan may promote sleep onset indirectly, by deactivating the awake state to set the stage for sleep onset. We suspect that this physiological deactivation may be readily reversed in experimental situations conducive to arousal, unlike the depressant effects of most sedative-hypnotics. While the "naturalness" of these deactivating effects and their probable reversibility may be viewed as l-tryptophan's strength as a hypnotic, they may also underlie its fundamental weakness, for its effectiveness may be influenced by the arousal state of the subject ingesting the drug as well as the conduciveness of the environment to sleep. Such reversibility may account for Broadhurst's finding (1977) that l-tryptophan (2 g) does not significantly slow reaction time as do barbiturate hypnotics in tasks performed 2 hours after administration and may further account for inconsistencies in the previously cited studies on sleep latency.

The results reported here are of importance in that they demonstrate the sleep-facilitating role of l-tryptophan in subjects who do not usually nap at the scheduled sleep times used in this study. The implication is that l-tryptophan is adequately deactivating to facilitate sleep onset at clock times other than normal bedtimes, particularly when the environment is conducive to sleep. Thus, l-tryptophan may be useful in alleviating sleep onset difficulties produced by time zone changes and fragmented sleep schedules, as well as in the clinical management of some nighttime sleep-onset insomniacs.

REFERENCES

Adam, K. & Oswald, I. One gram of l-tryptophan fails to alter the time taken to fall asleep. *Neuropharmacology*, 1979, 18:1025-1027.

Azmitia, Jr., E.C. & McEwen, B.S. Corticosterone regulation of tryptophan hydroxylase in midbrain of the rat. *Science*, 1969, 166:1274-1276.

Březinová, V., Loudon, J., & Oswald, I. Tryptophan and sleep. *Lancet*, 1972, 2:1086-1087.

Broadhurst, A.D. Tryptophan and sedation. Presented at 2nd Conference of I.S.T.R.Y., University of Wisconsin, Madison, Wisconsin, August 10-12, 1977.

Brown, C.C., Horrom, N.J., & Wagman, A.M.I. Effects of l-tryptophan on sleep onset insomniacs. *Waking & Sleeping*, 1979, 3:101-108.

Carskadon, M.A. & Dement, W.C. Sleep studies on a 90-minute day. *Electroencephalography & Clinical Neurophysiology*, 1975, 39:145-155.

Cooper, A.J. Tryptophan antidepressant 'physiological sedative': fact or fancy? *Psychopharmacology*, 1979, 61:97-102.

Curzon, G. & Knott, P.J. Fatty acids in the disposition of tryptophan. In: Aromatic Amino Acids in the Brain, Ciba Foundation Symposium 22, Elsevier, Amsterdam, 1974: 217-229.

Domino, D.F. & Krause, R.R. Plasma tryptophan tolerance curves in drug free normal controls, schizophrenic patients and prisoner volunteers. *Journal of Psychiatric Research*, 1974, 10:247-261.

Fernstrom, J.D. & Jacoby, J.H. The interaction of diet and drugs in modifying brain serotonin metabolism. *General Pharmacology*, 1975, 6:253-258.

Fernstrom, J.D. & Wurtman, R.J. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science*, 1972, 178:414-416.

Gessa, G.L. & Tagliamonte, A. Serum free tryptophan: control of brain concentrations of tryptophan and of synthesis of 5 hydroxytryptamine. In: Aromatic Amino Acids in the Brain, Ciba Foundation Symposium 22, Elsevier, Amsterdam, 1974: 207-216.

Globus, G.G. Rapid eye movement cycle in real time. *Archives of General Psychiatry*, 1966, 15:654-659.

Greenwood, M.H., Lader, M.H., Kantameneni, B.D., & Curzon, G. The acute effects of oral(-)-tryptophan in human subjects. *British Journal of Clinical Pharmacology*, 1975, 2:165-172.

Griffiths, W.J., Lester, B.K., Coulter, J.D., & Williams, H.L. Tryptophan and sleep in young adults. *Psychophysiology*, 1972, 9:345-356.

Hardeland, R. Circadian rhythmicity and regulation of enzymes of tryptophan metabolism in rat liver and kidney. *Zeitschrift fur Vergleichende Physiologie*, 1969, 63:119-129.

Hartmann, E. Dreaming sleep (the D-state) and the menstrual cycle. *Journal of Nervous & Mental Disease*, 1966, 143:406-416.

Hartmann, E.L. The effect of l-tryptophan on the sleep-dream cycle in man. *Psychonomic Science*, 1967, 8:479-480.

Hartmann, E., Chung, R., & Chien, C.-P. L-tryptophane and sleep. *Psychopharmacologia (Berlin)*, 1971, 19:114-127.

Hartmann, E., Cravens, J., & List, S. Hypnotic effects of l-tryptophan. *Archives of General Psychiatry*, 1974, 31:394-397.

Hartmann, E. & Elion, R. The insomnia of 'sleeping in a strange place': effects of l-tryptophane. *Psychopharmacology*, 1977, 53:131-133.

Hartmann, E. & Spinweber, C.L. Sleep induced by l-tryptophan. Effect of dosages within the normal dietary intake. *Journal of Nervous & Mental Disease*, 1979, 167:497-499.

Hartmann, E., Spinweber, C.L., & Ware, C. L-tryptophane, l-leucine, and placebo: effects on subjective alertness. *Sleep Research*, 1976, 5:57.

Hoddes, E., Zarcone, V., Smythe, H., Phillips, R., & Dement, W.C. Quantification of sleepiness: a new approach. *Psychophysiology*, 1973, 10:431-436.

Jacobs, B.L. & Jones, B.E. The role of central monoamine and acetylcholine systems in sleep-wakefulness states: mediation or modulation? In: L.L. Butcher (Ed.), Cholinergic-Monoaminergic Interactions in the Brain. Academic Press, New York, 1977: 271-290.

Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. *Ergebnisse der Physiologie*, 1972, 64:166-307.

Kelley, J., Laughlin, E., Carpenter, S., Simmons, J., Cidoric, K., & Lentz, R. A study of ninety-minute sleep cycles. *Stanford Review*, 1973, 3:1-5.

Knox, W.E. The regulation of tryptophan pyrrolase activity by tryptophan. *Advances in Enzyme Regulation*, 1966, 4:287-297.

Knox, W.E., Piras, M.M., & Tokuyama, K. Induction of tryptophan pyrrolase in rat liver by physiological amounts of hydrocortisone and secreted glucocorticoids. *Enzymologia Biologica et Clinica*, 1966, 7:1-10.

Moir, A.T.B. & Eccleston, D. The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. *Journal of Neurochemistry*, 1968, 15:1093-1108.

Moses, J.M., Hord, D.J., Lubin, A., Johnson, L.C., & Naitoh, P. Dynamics of nap sleep during a 40 hour period. *Electroencephalography & Clinical Neurophysiology*, 1975, 39:627-633.

Mouret, J. & Coindet, J. Polygraphic evidence against a critical role of the raphe nuclei in sleep in the rat. *Brain Research*, 1980, 186:273-287.

Nicholson, A.N. & Stone, B.M. L-tryptophan and sleep in healthy man. *Electroencephalography & Clinical Neurophysiology*, 1979, 47:539-545.

O'Hanlon, J.F. & Beatty, J. Concurrence of electroencephalographic and performance changes during a simulated radar watch and some implications for the arousal theory of vigilance. In: R.R. Mackie (Ed.), Vigilance: Theory, Operational Performance, and Physiological Correlates. Plenum Press, New York, 1977: 189-201.

Oswald, I., Ashcroft, G.W., Berger, R.J., Eccleston, D., Evans, J.I., & Thacore, V.R. Some experiments in the chemistry of normal sleep. *British Journal of Psychiatry*, 1966, 112:391-399.

Rechtschaffen, A. & Kales, A. (Eds.). A Manual of Standardized Terminology, Techniques and Scoring System for Sleep Stages of Human Subjects. Publich Health Service Publ. 204, U.S. Government Printing Office, Washington, D.C., 1968.

Rougeul, A., Corvisier, J. et Letalle, A. Rythmes electrocorticaux caracteristiques de l'installation du sommeil naturel chez le chat. Leurs rapports avec le comportement moteur. *Electroencephalography & Clinical Neurophysiology*, 1974, 37:41-57.

Small, J.G., Milstein, V., & Golay, S. L-tryptophan and other agents for sleep EEG. *Clinical Electroencephalography*, 1979, 10:60-68.

Spinweber, C.L. L-tryptophan, l-leucine and subjective alertness: A dietary study. Paper presented to the Association for the Psychophysiological Study of Sleep, Houston, 1977.

Spinweber, C.L. Daytime effects of l-tryptophan. *Psychopharmacology Bulletin*, 1981, 17:81-82.

Tagliamonte, A., Biggio, G., Vargiu, L., & Gessa, G.L. Free tryptophan in serum controls brain tryptophan level and serotonin synthesis. *Life Sciences, Part II*, 1973, 12:277-287.

Thayer, R.E. Measurement of activation through self-report. *Psychological Reports*, 1967, 20:663-678.

Thayer, R.E. Toward a psychological theory of multidimensional activation (arousal). *Motivation & Emotion*, 1978, 2:1-34.

Trulson, M.E. & Jacobs, B.L. Raphe unit activity in freely moving cats: correlation with level of behavioral arousal. *Brain Research*, 1979, 163:135-150.

Ursin, R. The effects of 5-hydroxytryptophan and l-tryptophan on wakefulness and sleep patterns in the cat. *Brain Research*, 1976, 106:105-115.

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(U) The effects of l-tryptophan (4 g) on the waking EEG and daytime sleep were studied in a group of 20 normal adults. Subjects were assigned to a morning or afternoon group, and data were collected on two occasions, after l-tryptophan and after placebo, assigned in a counter-balanced order. L-tryptophan significantly reduced sleep latency without altering nap sleep stages and elevated plasma total and free tryptophan levels. EEGs were digitized on-line and later

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20. ABSTRACT (continued)

analyzed for changes in five frequency bands: 16–40 c/sec (beta), 13.0–15.5 c/sec (sigma), 8.0–12.5 c/sec (alpha), 4.0–7.5 c/sec (theta) and 0.5–3.5 c/sec (delta). During waking EEGs, l-tryptophan significantly in eased alpha time, theta time, and theta intensity and significantly decreased alpha frequency. No wave bands were altered during sleep. L-tryptophan is an effective daytime hypnotic which can facilitate sleep onset at clock times which do not coincide with biological sleep times. The hypnotic effects may be mediated by lowering arousal level during the awake state, thus setting the stage for more rapid sleep onset.