Table 1. Concentrations of organic phosphate insecticides required for 50 percent inhibition of propanil-hydrolyzing enzyme in rice plants (PHE) and acetylcholinesterase (AChE.).

Compound	Concentration (mole/liter) for 50 percent inhibition of	
	PHE	AChE
Parathion	2.4×10^{-5}	2.0×10^{-5} (*9*)
Paraoxon	2.4×10^{-7}	2.0×10^{-9} (*9*)
Sumithion	3.4×10^{-4}	2.3×10^{-4} (*10*)
Sumioxon	1.5×10^{-6}	1.17×10^{-7} (*10*)

case of insects, the conversion of parathion to the more toxic paraoxon is inhibited by such materials as piperonyl butoxide, sulfoxide, and others (*8*). In the case of the rice enzyme, piperonyl butoxide had no significant effect on the inhibitory activity by parathion to the propanil detoxifying enzyme.

In the second experiment, rice plants were cultivated in a 500-ml plastic beaker filled with rice paddy soil, treated wtih 100 mg of ammonium sulfate per pot. Fifteen rice seeds were sown in each pot, covered with 2 mm of fine soil, and then incubated in a growth chamber at 30°C for 12 hours and 20°C during the night. To prevent flowering, two fluorescent lamps were maintained even at night. When rice reached the 2½-leaf stage, an emulsion of propanil at 0.3 percent was applied. Before dilution of the herbicide the insecticides were added to the emulsifiable concentrate of propanil at one-tenth the concentration of the propanil itself. As a further check, plants were treated with a diluted solution of the emulsifying agent and solvents used in formulating the propanil itself. Daily measurements were made of the fresh weight of the rice plants (Fig. 2).

The fresh weight of the rice plants was expressed as a percentage of the initial fresh weight at the time of treatment. Both the parathion and paraoxon used in this experiment enhanced the phytotoxicity of propanil toward rice plants. The paraoxon was significantly greater in its effect than parathion. At the termination of the experiment, plants treated with a combination of propanil and parathion still showed evidence of growth in that they formed new leaves, whereas the plot with propanil plus paraoxon was nearly completely dead.

Sumioxon, the oxygen analog, also had greater activity than sumithion, the parent compound. On the other hand, insecticides having no activity against acetylcholinesterase did not enhance phytotoxicity (*6*).

The two experiments described above may suggest some similarities between acetylcholinesterase and the propanil-hydrolyzing enzyme in rice plants. The inhibition of the propanil hydrolyzing enzyme by the organophosphorus and carbamate insecticides would appear to account for the phytotoxicity of the combination of propanil and insecticide to rice, since the tolerance of rice plants to the herbicide appears dependent upon hydrolysis.

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Sleep Patterns of the Monkey and Brain Serotonin Concentration: Effect of p-Chlorophenylalanine

Abstract. *The amount of time that monkeys* (Macaca mulatta) *slept was reduced after they were given* p-*chlorophenylalanine, a selective depletor of serotonin in animal tissues. The time spent in the rapid eye movement stage of sleep was unchanged, but the time in other sleep stages decreased. Seven regions of the brain had a 31 to 46 percent decrease in serotonin content; the concentration of cerebellar serotonin increased by 44 percent.*

Advances in the understanding of the physiology of sleep have led to the idea that there are two alternating, physiologically distinct sleep patterns during the normal sleep period of mammals (*1*). By behavioral, physiologic, electroencephalographic, and autonomic measurements, a short-term cycle of recurring patterns can be defined. Because of the general occurrence of this sleep cycle of rapid eye movement and nonrapid eye movement (REM-NREM) and because of studies implicating chemical control in the central nervous system (CNS) of these sleep states, a variety of naturally occurring substances, particularly presumed CNS transmitters, have been investigated (*2*). The demonstration that major alterations of the sleep patterns of man and animals occur when drugs are administered which affect the monoaminergic neurons has led investigators to postulate that serotonin and norepinephrine participate in the control of the sleep-waking cycle (*3*).

p-Chlorophenylalanine, which has been shown to be a potent and selective depletor of serotonin in animal tissues including the brain, acts by inhibiting hydroxylation of tryptophan, the first and rate-limiting reaction in the biosynthesis of serotonin (*4*). We now report the effects of administering *p*-chlorophenylalanine on sleep patterns (*5*) and on the regional concentrations of serotonin in brain tissue in monkeys

Silver disk electrodes for recording electroencephalographic (EEG) activity were placed on the dura mater overlying the brain of each monkey at six sites (through burr holes in the skull) and cemented in place with acrylic dental cement. Three additional electrodes were placed subcutaneously just lateral to the orbit overlying the temporalis muscle, bilaterally and above one eye. These recorded eye movements and activity of the temporalis muscle. The wires from the electrodes were attached to a connecting plug cemented to the skull. The monkeys, maintained in a restraining unit, were kept awake during the day and allowed to sleep from 10:00 p.m. to 6:00 a.m. During this sleep period, continuous EEG activity, eye movements, and temporalis muscle activity were recorded on an Offner model T machine. Direct observations of facial and eye movements were also

Table 1. Change in sleep pattern of monkeys after administration of *p*-chlorophenylalanine. Values in columns 3 through 6 are expressed as the mean percentage of the total time allowed for sleep (8 hours) for 3 nights before (pre) and 3 nights after (post) administration of drug. The values in columns 7 and 8 are the ratios of NREM sleep time of stages I plus II to stages III plus IV for 2 nights before and 2 nights after the drug was given.

Monkey	Dose (mg/kg)	Response (percentage of 8-hour recording time)				(I + II): (III + IV)	
		Awake		REM sleep		Pre	Post
		Pre	Post	Pre	Post		
		p-Chlorophenylalanine					
4 I	330	17	23	12	7		
5 I	330	11	24	18	14		
7 I	600	24	51	3	7	4	8
8 I	700	19	38	15	15	4	12
9 I	700	17	60	9	13	5	15
10 I	1000	19	35	13	11	2	4
13 I	800	21	42	14	11	1	10
		DL-*Alanine*					
11 1	800	28	23	11	19		
12 1	800	11	14	15	16		

continuously monitored by closed circuit television.

Approximately 1 week after surgery, the animal's sleep pattern was recorded for four successive nights. Between 30 and 40 hours after the end of the fourth base-line recording session, *p*-chlorophenylalanine (*6*) was injected intraperitoneally (six monkeys) and by a nasogastric tube into the stomach (one monkey) (Table 1, see dose schedule). Two additional animals were given an intraperitoneal injection of DL-alanine. Approximately 30 hours after the drug was administered, the sleep pattern was recorded on five successive nights (10:00 p.m. to 6:00 a.m.) as described for the base-line period. For each 8-hour record, three physiologic states were distinguished: awake, REM sleep, and NREM sleep (*5*). The animal was considered to be awake when its eyes were open, the EEG pattern had low voltage and fast frequency, and the record of temporalis muscle activity had high amplitude. Rapid eye movement sleep was defined by a low voltage, fast-frequency EEG pattern, absent tonic muscle activity, and rapid conjugate eye movements present with partially or fully closed lids. The NREM sleep consisted of partially or fully closed lids with no rapid eye movements, high voltage, slow waves, K-complexes and 14-cycle-per-second spindles, and a low to moderate muscle activity record. The NREM sleep was further divided into stages I, II, III, and IV, according to criteria described (*5*, *7*).

The data are expressed as the percentage of the total time for each of the three states (Table 1) for 3 nights just preceding and 3 nights after administration of the drug. All monkeys given *p*-chlorophenylalanine slept less. The high-dose group slept one-half as much after drug administration. No difference in waking time was found when DL-alanine was substituted for *p*-chlorophenylalanine. Because of the possibility of nonspecific change in sleep pattern due to intraperitoneal injection, one monkey received the *p*-chlorophenylalanine by stomach tube. A similar increase in waking time occurred in this animal as well. The animals did not appear to have any alteration in aggressiveness, alertness, or appetite for food, nor was any specific neurological change noted during the daytime waking period after the drug was given.

Table 2. Serotonin content of regional areas of monkey brain. Results are given as mean and standard deviations of single tissue specimens from each of four animals. *p*-CPA, *p*-chlorophenylalanine.

Regional areas	Untreated (μg/g)	After *p*-CPA (μg/g)	Reduction (%)
Midbrain	1.03 ± .12	0.60 ± .16	42
Hypothalamus	0.76 ± .10	.41 ± .09	46
Pons	.71 ± .10	.44 ± .13	38
Medulla	.64 ± .09	.39 ± .11	39
Thalamus	.39 ± .08	.27 ± .07	31
Hippocampus	.33 ± .05	.20 ± .05	39
Neocortex	.28 ± .05	.19 ± .02	32
Cerebellum	.18 ± .03	.26 ± .05	(− 44)

The decrease in total sleep time after administration of *p*-chlorophenylalanine was at the expense of NREM sleep and not of REM sleep (Table 1). No consistent change in total REM sleep time was found. The percentage of REM time to total sleep time was therefore increased. In order to determine whether a selective change in NREM sleep stages had occurred, a value for the ratio of the number of minutes spent in stage I plus stage II to the number in stage III plus stage IV was calculated for the five monkeys given a dose of 600 mg/kg or more for the two sleep periods just before and just after the drug was administered. In all cases, an increased value for the ratio was found after the drug was given, indicating that sleep for stage III plus stage IV was inhibited to a greater degree than sleep during stage I plus stage II.

Regional alterations in serotonin concentration after administration of *p*-chlorophenylalanine were studied in eight CNS areas in each of eight monkeys (four experimental and four controls). Of the four treated monkeys, three (8I, 9I, 10I) were given *p*-chlorophenylalanine intraperitoneally 9 days after a previous injection used for sleep analysis, and the fourth (4I) received the drug 4 weeks after the previous injection. Three of the control monkeys (2I, 5I, 7I) had received an injection of *p*-chlorophenylalanine 31, 75, and 75 days before being killed, and one control animal had never received *p*-chlorophenylalanine. Since the results of studies in rats indicate that the peak decrease in CNS serotonin concentrations occurs on the 2nd and 3rd days after injection, the experimental animals were killed between 46 and 70 hours after *p*-chlorophenylalanine was given (*4*). Nembutal was given intravenously (30 mg/kg), and perfusion of the brain with normal saline was begun within 10 minutes of anesthesia. After thoractomy, the descending aorta was clamped, the right atrium was incised, and perfusion was accomplished by needle puncture of the left ventricle of the heart. After perfusion, the whole brain was removed rapidly, and the regions to be studied were cut, weighed, and frozen. The time from removal of the brain to freezing was 15 to 30 minutes; the total time from onset of perfusion to freezing of tissues was 45 minutes or less. Serotonin concentrations were determined on 800 to 1000 mg of tissue (*8*).

Normal values for eight regional

areas (Table 2) show that all contained significant quantities of serotonin. After treatment with *p*-chlorophenylalanine, average values for serotonin content decreased for all areas except the cerebellum, where there was an increase of 44 percent. The significance of this increase is not understood. Although gross depression of serotonin concentration provides no information on the relative effect of the drug on metabolically stable and labile pools, or on the relation of these pool sizes to site of action, it is clear that a very large depression of serotonin content (greater than 50 percent) in the upper brain stem is not necessary to produce alterations in the sleep pattern of the monkey. It is probable that more pronounced depression of serotonin concentration would produce more profound alterations in sleep pattern, since the animal (9I) in which this alteration was most pronounced showed a great decrease in serotonin content.

These findings support the notion that serotonin-containing neurons of the CNS may play a role in the control of sleep. Our finding of a selective decrease of NREM sleep without a significant change in REM sleep time indicates that the two sleep states can be dissociated and suggests that serotonin may be specifically related to the NREM sleep phase. A study (*3*) of the effect of *p*-chlorophenylalanine on the sleep patterns of cats showed a profound decrease in sleep time. However, in that study both phases of sleep decreased 48 hours after the second of two injections with complete insomnia lasting for 2 days. During the recovery phase, a phasic indicator of REM sleep (pontogeniculo-occipital spikes) returned first, with progressive return of both phases of sleep thereafter. Since regional brain serotonin concentrations were not reported, the differing results cannot be compared. Studies in which lesions destroying the raphe system in the midline of the upper brain stem, a region rich in serotonin neurons (*10*), have shown a linear relation between decrease in sleep and serotonin concentration in several regions of the brain. It is of interest that the greatest decrease in tissue serotonin in the monkey after *p*-chlorophenylalanine occurred in comparable rostral brain stem regions.

The degree of serotonin depletion in our monkeys was not as great as reported for lower mammals, despite a higher dosage of drug (*4*). The NREM sleep pattern of the monkey is much closer to that of man than is that of the cat. This similarity of monkey and man would be consistent with the failure to observe insomnia as a striking symptom when the drug was administered to normal man and to patients (*9*).

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Hippocampal Correlates of Aversive Midbrain Stimulation

Abstract. *Hippocampal synchronization during aversive dorsal midbrain stimulation was observed in rats both in a conditioning procedure and under* d-*tubocurarine paralysis. The results restrict the generality of previous reports which correlated hippocampal synchronization and desynchronization with approach and withdrawal behavior, respectively. Relative to the condition of free movement, curarization reduced the frequency of both "spontaneous" and dorsal midbrain-evoked synchronization, thus suggesting possible direct and indirect effects of* d-*tubocurarine on subcortical structures.*

Since Green and Arduini's (*1*) extensive analysis of theta patterns in rabbit hippocampus, several attempts have been made to determine the behavioral correlates of hippocampal activity. One characteristic of hippocampal activity as examined by electroencephalographic techniques is the occurrence of distinct shifts between synchronization and desynchronization. An important series of investigations by Grastyan and coworkers (*2*, *3*) has suggested that this biphasic activity of the hippocampus is directly related to motivational mechanisms such that "there is a strict correlation between approach behavior and hippocampal theta rhythm on the one hand and withdrawal behavior and desynchronization on the other" (*2*, p. 91). These investigators prefer the terms "pull" and "push" to describe the "two irreducible behavioral patterns during which the animal moves toward (pull) or away from (push) an object or environmental stimulus" (*3*). The behavioral data suggest that "pull" and "push" behaviors occur, in the terminology of American behavioral analysis, during rewarding and aversive situations respectively.

In our laboratory (*4*) rewarding hypothalamic stimulation was recently found to produce concomitant hippocampal synchronization, supporting the hypothesis of Grastyan *et al.* (*2*). However, rebound effects reported by these workers were not found. In addition, other studies of hippocampal activity during eating, drinking, and grooming disclosed desynchronization, not synchronization, during such approach-type behavior (*5*). These latter results suggested the possible need to modify the correlation between synchronization and reward. Since the strict relation of desynchronization and aversion could also be questioned, the present experiment examined the generality of the desynchronization-aversion correlation by observation of hippocampal activity during stimulation of dorsal midbrain, an area shown by Olds (*6*) to have aversive properties.

Five adult albino rats were implanted