EFFECTS OF PARA-CHLOROPHENYLALANINE (A SEROTONIN DEPLETOR) ON CONDITIONED ALTERNATION IN A CHOICE CHAMBER

A Dissertation

Presented to

the Faculty of the Department of Psychology

University of Houston

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

Robert A. McFarlain

August, 1970

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ABSTRACT

The effects of the serotonin depleting drug para-chlorophenylalanine (P-CPA) on conditioned alternation in the U-maze were examined. Rats given P-CPA and nondrugged rats received reward contingent upon alternation. Every S received reward on the first trial of each day whether S chose the left or right maze arm. On trials two and three, contingent reward Ss had to choose the arm opposite that chosen on the previous trial to obtain reward.

To evaluate the effectiveness of the reward contingency, the "spontaneous" alternation level was determined by observing the alternation behavior of a group of nondrugged Ss yoked to some of the contingent reward nondrugged control Ss. A yoked rat received reward only if its contingent reward counterpart alternated and received reward. Training was given for 30 days with a 30 min. ITI.

Because there was no effect of the reward contingency on alternation scores after 30 days of training with a 30 min. ITI, the ITI was reduced to 30 sec. for an additional 20 days of training. The reward contingent Ss did perform at higher alternation levels than the yoked rats when the ITI was reduced to 30 sec. There was no difference in alternation performance between the drugged and nondrugged contingent reward groups during the experiment. The failure to find a P-CPA effect on conditioned alternation during the short ITI phase of the experiment implies that serotonin does not have an important role in memory processes.

When the rate of development of rapid running in the maze was examined by comparing the first trial latencies of drugged and control Ss, the drugged Ss evidenced faster acquisition than the non-drugged Ss but asymptotic running times were unaffected by P-CPA.

P-CPA was shown to reduce food intake and body weight.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The current interest of psychologists in serotonin is based upon the findings that serotonin exists in high concentrations in the brain (Amin, Crawford, and Gaddum, 1954), and that some chemicals which produce dramatic changes in mental and behavioral states also produce changes in the functional levels of serotonin in the brain. Reserpine causes sedation and depletion of brain serotonin (Shore, Pletscher, Tomich, Carlsson, Kuntzman, and Brodie, 1966); LSD-25 causes hallucinations and antagonism of serotonin (Gaddum, 1956), a functional depletion.

In 1959, Kety reviewed evidence contrary to the notion that LSD-25 hallucinations and reserpine sedation are based upon serotonin changes in the brain. Reserpine has been shown to lower both serotonin (5HT) and norepinephrine (NE) brain levels (Woolley and Van Der Hoeven, 1963); and Kety cited the findings of Carlsson, Lindquist, and Magnusson (1957) that 3, 4-dihydroxy-phenylalanine, a precursor of NE, diminished reserpine sedation in mice and rabbits while the precursor of serotonin, 5-hydroxytryptophan (5HTP) did not diminish sedation. These findings suggest that reserpine sedation may be due to decreased NE rather than to decreased 5HT levels. Kety also cited Rothlin's (1957) observation that the compound 2-bromo-lysergic acid diethylamide does not produce hallucinations even though it shows greater antiserotonin action than

LSD-25, indicating that the mental changes caused by LSD-25 are not due to antagonism of 5HT mechanisms.

Even though the speculations that LSD-25 and reserpine effects are due to changes in brain serotonin have not been supported by subsequent research, an hypothesis of the role of brain serotonin based on these speculations has received experimental support. Brodie and Shore (1956) suggested that serotonin is a neural transmitter substance. They further hypothesized that the serotonergic neurons mediate parasympathetic-like reactions that result in behavioral inactivity.

Considerable experimental evidence has accumulated which supports the hypothesized neural transmitter role for serotonin. Studies of the rat brain indicate that serotonin exists in a specific neural system originating in the midbrain raphe' nucleus (Dahlström and Fuxe, 1964) and projecting through the medial forebrain bundle (Heller and Moore, 1968) to eventually innervate virtually all of the telencephalon. The specificity of this neural system suggests that brain 5HT does not have merely a general metabolic function in CNS neurons. The concentrations of 5HT in the rostral portion of this system have been shown to increase following electrical stimulation of the midbrain raphe' (Aghajanian, Rosecrans, and Sheard, 1967). More direct evidence for a synaptic transmitter role for serotonin is the fact that 5HT and its precursor, 5HTP, when injected intravenously, intraperitoneally, or intracellularly, cause changes in single unit firing rates in cats (Krnjevic and Phillis, 1963a; Weight and Salmoiraghi, 1968; Eidelberg, Goldstein, and Deza, 1967) and snails (Gerschenfeld and Stefani, 1968).

Furthermore, Anden, Jukes, and Lundberg (1964) have shown that intravenous injections of 5HTP result in inhibition of afferent potentials and excitation of efferent potentials in cat spinal cord. These findings are all consistent with the hypothesized neural transmitter function of 5HT.

If serotonin is a neural transmitter in the brain, manipulation of 5HT levels should result in changes in behavior, especially if serotonin is depleted. There is a method of producing an impressive decrease in brain 5HT levels. Administration of the drug dl para-chlorophenylalanine (P-CPA) produces a 90% reduction in brain serotonin.

Surprisingly, lowering serotonin levels with P-CPA has been shown by a number of investigators to improve performance in certain learning tasks. The improvement of behavior in these situations is not necessarily due to changes in associative mechanisms. Every case of behavior facilitation observed can be accounted for in terms of lowered sensory thresholds rather than changes in associative processes. But if the observed facilitation of performance following P-CPA treatment is due to changes in associative mechanisms, a similar facilitation of memory processes may occur.

Statement of the Problem

The present experiment was designed to examine the effects of P-CPA on a behavior that is controlled by an internal cue, a "memory." Any effect on this behavior by P-CPA treatment can be attributed to changes in associative or memory processes rather than changes in sensory thresholds. The method used was developed by Bolles, Petrinovich, and associates to study memory (Petrinovich and Bolles, 1954; Petrinovich and

Bolles, 1957; Bolles and Petrinovich, 1956; Petrinovich, Bradford, and McGaugh, 1965). The method involves a two alternative choice situation in which rats may turn either right or left at a choice point. The Ss are always rewarded on the first trial of each day. However, to receive reward on subsequent trials, S has to choose the response alternative other than the one chosen on the previous trial, whether the previous response was rewarded or not. Successful performance depends upon learning the rule "Go to the side opposite that chosen on the previous trial," and upon the ability to remember which side of the maze was chosen on the previous trial. This is true, of course, only if there is no external stimulus event, such as an odor, available at the time of the choice which indicates to the Ss either its previous choice or the present location of the reward.

One of the major differences between the method employed in this experiment and the method of Bolles, Petrinovich, and associates is that while their apparatus has been an elevated T-maze, the maze used in the present study is an enclosed U-maze equipped with two exhaust fans which continually pull air into the maze at the start point and out of the maze at the two goal boxes. These fans have been used effectively in previous studies at this laboratory to eliminate odor cues. Because the arms of the elevated T-maze used by Petrinovich, Bolles, and associates were only 61 cm. long, it is possible that the performance they attributed to memory was actually based on either odor cues left by Ss on the previous trial, or odors from the food in the goal box. The fans eliminate both of these possibilities in this experiment.

CHAPTER II

LITERATURE

The present experiment is an examination of the effects of the serotonin-depleting drug para-chlorophenylalanine (P-CPA) on memory in adult rats. Only the previous observations of adult rats or mice following serotonin depletion with P-CPA treatment are reviewed in detail. The studies examining the effects of serotonin depletion by feeding excess phenylalanine and those experiments using neonates or species other than rats or mice are not extensively reviewed and are mentioned only to point out current trends of interest or to indicate the generality of research findings from rodent experiments with P-CPA.

The Effect of P-CPA on Serotonin and Norepinephrine

It is generally assumed that the effects of P-CPA on behavior are due to the dramatic decrease in serotonin that results from P-CPA treatment. However, changes in serotonin levels are not the only consequences of P-CPA treatment. P-CPA has been shown to increase levels of the amino acid phenylalanine in plasma (Lipton, Guroff, and Udenfriend, 1967) and in the brain (Jequier, Louenberg, and Sjoerdsma, 1967). The excess phenylalanine and its metabolites may possibly produce as yet unknown abnormalities in brain chemistry and physiology. Even though the additional biochemical effects of P-CPA treatment may confuse the interpretation of P-CPA produced behavior changes, the fact that P-CPA treatment results in such a large reduction in brain 5HT makes P-CPA the best

tool available for studying the role of serotonergic fibers in the determination of behavior.

Koe and Weissman (1966) were the first to observe that the administration of dl para-chlorophenylalanine produces a decrease in brain serotonin of up to 90%. Koe and Weissman found that P-CPA treatment depleted brain NE only slightly. But there is still some question whether or not P-CPA produces significant alterations of brain norepinephrine levels.

Welch and Welch (1968) gave mice a single i.p. injection of P-CPA (360 mg/kg) and found that brain NE levels were significantly reduced 10 min. after the injection. Chrusciel and Herman (1969), also using mice, found increases in brain NE within 3 hr. after a single 50 mg/kg dose of P-CPA but decreases in brain NE if brain assays were done 24 hr. after the last of three daily doses of 320 mg/kg. Volicer (1969) gave rats a single 400 mg/kg treatment with P-CPA and found that NE levels were unchanged 72 hr. after the P-CPA administration. Schlesinger, Schreiber, Grìek, and Henry (1969) gave mice a single i.p. administration of P-CPA (320 mg/kg) and also observed no effect on NE levels 72 hr. later. Apparently, P-CPA does not consistently alter brain NE levels.

Performance in Learning or Memory Tasks Following P-CPA Treatment

While P-CPA produces drastic depletion of serotonin in both neonate and adult animals, learning deficits following P-CPA treatment reliably occur only when the drug is given to neonates. For example, Kilbey (1969) found that daily P-CPA treatment of neonate rats beginning within

8 hr. after birth impaired the development of a classically conditioned leg flexion response.

An experimental examination of the effects of subcutaneous injections of P-CPA when injections were begun at different ages was performed by Watt and Martin (1969). They recorded performance in a six unit water maze. Rat Ss had to swim through cold water to escape to a warmed platform. It was found that if drug treatment was instituted within 14 days after birth, P-CPA produced a marked impairment of maze performance. If P-CPA treatment was not begun until post partum day 32, and Ss were then treated with daily doses as high as 200 mg/kg, the P-CPA treated Ss made fewer mean errors ($\bar{X}$ = 48) than the control Ss ($\bar{X}$ = 67), but the difference was not statistically significant.

Schlesinger, Schreiber, and Pryor (1968) observed better performance by P-CPA treated rats than by controls in an active pole climb avoidance task if the P-CPA was given in a single 320 mg/kg dose to mature Ss. However the drug interfered with learning if it had been administered every third day beginning 24 hr. after birth for 40 days prior to the learning task. The reports of behavioral deficits following P-CPA treatment of neonates may reflect a general debilitating effect of early and prolonged P-CPA treatment because of an interference with certain physical growth factors. This interpretation has been previously offered as a possible explanation of the learning deficits observed following administration of excess phenylalanine in the diets of neonates (Karrer and Cahilly, 1965).

The reports of Watt and Martin (1969) and Schlesinger, Schreiber, and Pryor (1968) that behavioral deficits occurred when P-CPA was administered to neonates but performance facilitation resulted when adult rats received P-CPA is relevant to the interpretation of P-CPA produced behavioral changes. The drug P-CPA produces serotonin depletion and phenylalanine elevation in both neonate and mature rats. Phenylalanine excesses may produce the retarded learning observed with neonates because excess phenylalanine retards the myelinization process (Waisman, Hable, Wang, and Akert, 1964). If interference with myelinization is the mechanism producing retardation in neonates, it would not be expected that the excess phenylalanine levels which result from P-CPA treatment would retard learning in adult rats because myelinization would be more complete. Behavioral changes in adult rats following P-CPA treatment would be more confidently attributed to the serotonin depletion that results from P-CPA treatment rather than to the excess phenylalanine and its metabolites which accompany the serotonin depletion.

Tenen was the first to observe that when P-CPA is administered to adult rats, facilitation of performance may occur. Tenen (1967) observed the rate of acquisition of a discriminative avoidance response 24 hr. after the third daily 100 mg/kg treatment with P-CPA. Adult rat Ss had to jump to a platform following an auditory CS to avoid shock. The drugged Ss learned the avoidance response more rapidly than control rats. The difference in learning rates (trials to criterion) between serotonin depleted and normal rats was not observed when the shock intensity was increased. Because Tenen also found that P-CPA produced

lower shock thresholds for jumping, he concluded that the drug effects on avoidance performance were due to lowered pain thresholds rather than changes in associative mechanisms.

Facilitation of learning following P-CPA treatment has also been observed by Brody (1968). He found, as did Tenen, that P-CPA facilitated learning to jump to a platform to avoid shock. Brody further found that P-CPA treatment resulted in faster learning of a passive step-down avoidance task. This latter finding is of special interest because it seems to rule out the possibility that the observed facilitation of active avoidance is due to hyperactivity. Hyperactivity would interfere with learning not to step down from a small platform onto an electrified grid. The effects of P-CPA on activity will be discussed later in this paper.

Stevens, Resnick, and Krus (1967) found that P-CPA facilitated performance in two brightness discrimination tasks. They used a Y-maze with water reward to assess the effects of 316 mg/kg doses of P-CPA given every third day on the learning of a position habit, a successive brightness discrimination, and a simultaneous brightness discrimination. They found that while P-CPA did not affect learning of the position habit, the drug did facilitate learning of both brightness discrimination tasks.

Tenen (1967) gave 100 mg/kg of P-CPA daily for three days, and 24 hr. after the third dose, trained adult rats in a T-maze. Tenen gave water rewards for the development of a position habit. Like Stevens, Resnick, and Krus, Tenen found that learning a position habit was not affected by the P-CPA treatment.

In an examination of P-CPA effects on two different avoidance tasks, Koe and Weissman (1965) found that a 178 mg/kg i.p. administration of P-CPA did not affect discriminative avoidance behavior when rats were tested 24 hr. after treatment. Sidman avoidance behavior of three well trained rats was also unaffected by three daily 100 mg/kg doses of P-CPA.

While all of the studies reviewed employing acute P-CPA treatments of mature rats revealed either no effect or improvement of learning in drugged Ss, Stevens, Fechter, and Resnick (1968) observed that acute P-CPA treatments resulted in retarded passive avoidance learning in adult rats. In this study, Ss given a single 316 mg/kg treatment with P-CPA took longer to stop running to an electrified water fount than nondrugged controls.

The results of recent studies of the effects of P-CPA on the conditioned emotional response and on passive avoidance behavior agree with the findings of Stevens, Fechter, and Resnick (1968) that P-CPA treatment interferes with these behaviors. Robichaud and Sledge (1969) trained six rats to bar press for milk on a VI schedule. They then presented a tone at 15 min. intervals. During the tone periods, every response resulted in a reinforcement and a shock. Very few responses were made when the tone was on. A 200 mg/kg dose of P-CPA abolished the behavioral suppression which usually occurred in response to the tone.

Geller and Blum (1969) also trained rat Ss to bar press for a liquid reinforcement. In a replication of the Robichaud and Sledge study, Geller and Blum again found that P-CPA reduced the suppression effects of a tone-signalled punishment period. Geller and Blum further

found that a 5HTP injection (15 mg/kg, 2 hr. before test) attenuated the P-CPA effect and response suppression was again observed to occur to the tone following the 5HTP injection.

Tenen (1967) had earlier found that behavioral suppression is attenuated by P-CPA. Tenen paired a light and tone with shock and then examined the effects of P-CPA on the "conditioned emotional response" (the suppression of drinking when the light and tone were presented). P-CPA treated Ss showed less disruption of drinking when the light and tone were presented than did the nondrugged Ss.

It is interesting to note that in all of the studies in which P-CPA interfered with the suppression of a behavior, liquid reinforcements were given for the behavior that was to be suppressed. P-CPA has been shown to increase water intake (Brody, 1969). It may be that the difficulty in suppressing a liquid reinforced response in P-CPA treated Ss is due to increased thirst motivation caused by the drug. In the one study examining the effects of P-CPA on the suppression of a behavior that was not rewarded with a liquid (Brody, 1968), P-CPA Ss evidenced faster suppression (passive avoidance learning) than control Ss.

Serotonin Contribution to ECS-Induced Amnesia

In addition to the demonstrations of an influence of the serotonin depletor P-CPA on learning or performance in learning tasks, Essman has implicated serotonin in the production of retrograde amnesia following electroconvulsive shock (ECS). Essman gave mice one training trial on a passive avoidance task. When Ss left a small chamber and entered an adjacent large chamber, they received electric foot shock and then ECS. In saline treated mice, ECS increased brain serotonin levels and pro-

duced retrograde amnesia; i.e., saline treated mice had short latencies for the punished response when they were tested 24 hr. later. But mice treated with the drugs amitriptyline, nialamide, or pipradol (Essman, 1968a) or with dimethoxy-aminoethyl-N-benzyl-M-methoxy-cinnamide hydrochloride (Essman, 1968b) did not show an increase in brain 5HT following ECS and retained the conditioned passive avoidance better than nondrugged Ss, as evidenced by the longer latencies of the drugged Ss when tested for retention.

Role of Serotonin in Controlling Sleep

There are many studies examining the effects of P-CPA treatment on behaviors other than those observed in learning or memory tasks. Recently Jouvet (1969) has reviewed a number of studies which suggest that serotonin has a role in the control of sleep. Jouvet and his associates have produced "total insomnia" in the cat by depleting brain serotonin with a single 400 mg/kg injection of P-CPA. Insomnia has also been produced by depleting brain 5HT neurosurgically by lesioning the midbrain raphe' nucleus. Following 5HT depletion by P-CPA, the resulting insomnia has been terminated by injecting 5HTP, the direct precursor of serotonin. These and related findings led Jouvet (1969) to hypothesize that 5HT is part of the normal biochemical mechanism controlling sleep, specifically slow wave sleep. A slightly more detailed but poorly substantiated hypothesis about the relation between 5HT and sleep has been offered by Torda (1968). She suggests that 5HT has its effects on sleep by inhibiting cholinergic synapses in the hippocampus.

In connection with the P-CPA produced insomnia in cats, it is interesting to note that when Cremata and Koe (1966) administered P-CPA to prison inmates, even in large doses, the Ss reported "tiredness." No mention was made of insomnia following P-CPA administration as has been described for cats similarly treated.

P-CPA-Induced Hypersexuality

Even more interesting than the sleep changes observed is the hypersexuality that results from P-CPA administration. Reports by Sheard (1969) and by Tagliamonte, Tagliamonte, Gessa, and Brodie (1969) describe abnormal sexual behavior (excessive mounting) in both male and female rats following i.p. administrations of P-CPA (100 and 320 mg/kg respectively). P-CPA even produced hypersexuality in 7 out of 12 pinealectomized rats in the Tagliamonte et. al. study. The observation of hypersexuality in pinealectomized Ss rules out the possibility that P-CPA-induced hypersexuality is caused by the reduction of the pineal hormones (e.g., melatonin) which themselves inhibit sexual behavior (McIsaac, Taborsky, and Farrell, 1964; Wurtman, Axelrod, and Chu, 1963). However, the facts that P-CPA induced 5HT depletion does increase sexual responsiveness and that injections of 5HTP, in combination with a monoamine oxidase inhibitor produce a complete absence of estrus in female rats (Meyerson, 1964) are rather convincing evidence for an involvement of brain serotonin in the control of sexual excitability.

Raphe' Stimulation and Behavior Suppression

The previously mentioned findings of Robichaud and Sledge (1969), Geller and Blum (1969), and Stevens, Fechter, and Resnick (1968) that

P-CPA reduces behavior suppression in passive avoidance tasks suggest that serotonin may have a general role in behavior suppression. If serotonin does mediate behavior suppression, we would expect suppression to occur when the serotonergic neural system is activated. Van Twyver, Fairchild, and Sterman (1969) stimulated the raphe' nucleus of cats with low frequency electrical pulses while the cats ran down an alley to milk. Pronounced behavioral inhibition was observed in more than one-half of the electrode placements in the raphe'. The finding of Van Twyver et. al. must be cautiously interpreted. When Sheard and Aghajanian (1968) electrically stimulated the raphe' neurons of rats with low frequency electrical pulses, they did not observe the behavioral inhibition Van Twyver et. al. found. Sheard and Aghajanian tested habituation to an air puff before, during, and after raphe' stimulation. They found that raphe' stimulation produced a recurrence of the previously habituated startle response to the air puff, and raphe' stimulation maintained the reinstituted startle response. The effect of raphe' stimulation was abolished by a 300 mg/kg i.p. dose of P-CPA. In the Sheard and Aghajanian study, gross visual examination of the Ss revealed no behavioral suppression during raphe' stimulation. Apparently there were only minor differences in the location and the frequency of stimulation between the studies by Van Twyver et. al. and by Sheard and Aghajanian. Differences in the species of the subjects or in the intensity of stimulation may have produced the divergent results.

Effects of P-CPA on Activity

Any hypothesis that serotonergic fibers normally contribute to behavioral suppression or inhibition would have to predict increases in activity following P-CPA treatment. Tenen (1967) measured the open field mobility of adult rats treated orally with P-CPA (100 mg/kg). The drug treated rats were more mobile in the open field situation and in a tambour-cage test for locomotion than were control Ss 24 hr. after the last of three daily 100 mg/kg doses of P-CPA. Chrusciel and Herman (1969) gave three daily doses of 320 mg/kg of P-CPA to mice and observed a similar increase in tambour-cage activity.

Stevens, Fechter, and Resnick (1968) studied ambulation of adult rats in an open field. A novel stimulus was located in the center of the field when Ss were tested. The experimenters gave a single i.p. injection (315 mg/kg) of P-CPA and tested for ambulation on the third, fourth, and fifth days following the injection. Stevens et. al. found that the drugged rats were less mobile than the nondrugged rats.

Other recent studies have also shown decreases in open field activity following P-CPA treatment. Volicer (1969) gave adult rats a single 400 mg/kg treatment with P-CPA and observed Ss in an open field for three days following the injections. P-CPA produced a decrease in mobility, and the effect was greatest on the third post-treatment day.

The P-CPA induced increase in ambulation observed by Tenen is difficult to reconcile with the decrease in ambulation following P-CPA treatment observed by Stevens, Fechter, and Resnick, and by Volicer. All three studies used adult rats. Tenen gave three daily 100 mg/kg

doses and observed behavior 24 hr. after the last drug treatment. Stevens et. al. and Volicer gave single, large doses of P-CPA (316 and 400 mg/kg respectively) and observed behavior over several post-treatment days. It seems unlikely that these procedural differences account for the opposite P-CPA effects in the studies.

Adding to the confusion is the report of Brody (1968). He recorded the open field ambulation of P-CPA treated rats three days after a single i.p. injection of 300 mg/kg under either normal or extra stimulation conditions. Control and P-CPA treated Ss in the extra stimulation condition were presented with light and white noise stimuli, both of which were controlled by a relay coil which was activated for .1 sec. every second. The P-CPA treated Ss were more active than controls in the extra stimulation condition but less active than the controls in the normal stimulation condition.

Brody's findings are interesting, but they make even more difficult the interpretation of previous activity experiment findings. On the basis of Brody's results, we would have expected the procedure of Stevens, Fechter, and Resnick to produce a P-CPA facilitation of mobility because Stevens et. al. used a stimulus-rich open field environment (e.g., they had an open plastic container filled with brass hardware in the middle of the field). However, Stevens et. al. found that P-CPA reduced mobility.

P-CPA Effects on Shock Thresholds

P-CPA has been shown to lower sensory thresholds in some situations. Tenen (1967) determined shock thresholds for rats by noting whether or not each S jumped or flinched when it received a foot shock of a certain intensity. Tenen found that P-CPA treated Ss (three daily 100 mg/kg doses) had lower shock thresholds than normal rats. Lints and Harvey (1969) also observed decreased shock thresholds in rats using the jump-flinch rating method following lesioning of the medial forebrain bundle, septal area, or dorsemedial tegmentum. Lesions in these areas were shown to lower brain serotonin levels.

P-CPA Effects on Intake of Quinine and Sucrose

Brody (1969) has noted that rat Ss treated with 100 mg/kg of P-CPA consumed less quinine solution than control Ss when only a quinine solution was available for drinking. Similar changes in the ingestion of sapid substances following P-CPA administration have been observed by Nance and Kilbey (1970). In the Nance and Kilbey study, rats treated with daily 100 mg/kg doses of P-CPA consumed more of a low concentration sucrose solution than normal rats.

P-CPA Effects on Seizure Susceptibility

The effects of P-CPA treatment of mice on audiogenic seizure susceptibility were examined by Schlesinger, Schreiber, Griek, and Henry (1969). Schlesinger et. al. gave either acute or chronic P-CPA i.p. injections. They then recorded the incidence of wild running and of clonic, tonic, and lethal seizures of two highly inbred strains of mice and of hybrid mice when a 102 db. noise (from a bell) was sounded for

90 seconds. Hybrid mice showed a statistically significant increase in seizure susceptibility as a result of P-CPA treatment.

Summary

In summary, the learning studies showing facilitation of learning or performance have all used an external stimulus event (shock, cold water, or brightness differences) to motivate the behavior or to indicate the correct response. Studies in which the response is not controlled by shock, and an external stimulus does not indicate the correct response (position habit learning) have reported no P-CPA effect on learning.

The alternation task used in this experiment requires the utilization of internal rather than external cues. Previous studies in which the behavior was controlled by internal cues (position habit learning) observed no P-CPA effect on learning. Therefore, any P-CPA effects on alternation can reasonably be attributed to memory rather than learning changes.

On the basis of facilitation of performance in learning tasks and the suggestion of Essman (1968a) that "a portion of the amnesic effect of electroshock can be accounted for by an increase in brain serotonin that it produces during the consolidation period" (p. 265), it was expected that treatment with the serotonin depletor P-CPA might enhance the memory process and a behavior thought to be dependent upon memory.

CHAPTER III

METHOD

Subjects

The subjects (Ss) were 70 experimentally naive, albino male rats purchased from the Holtzman Company, Madison, Wisconsin. Ss were 210 days old at the beginning of the experiment.

Apparatus

The U-maze used is pictured in Figure 1. It has a 50 cm. main stem that is 10 cm. wide. The choice point at the end of the main stem is 14 cm. long and 38 cm. wide. On either side of the choice point is a goal box which is 35.5 cm. long and 10 cm. wide. The maze is 14 cm. high throughout. The floor is of hardware cloth and is painted a flat grey, as is the rest of the maze except the clear plexiglas roof. Each goal box contains a sliding food tray 2.8 cm. deep, 3 cm. wide and 21.6 cm. long which is divided into two 10.8 cm. long sections. Each section of the tray can be aligned so that the other portion is not accessible. One section of the tray was used for all rewards and the other for all nonrewards. A 6.4 cm. high wooden barrier mounted immediately in front of the food tray prevents S from viewing the food cup before entering the goal box.

Two photoelectric units mounted 12.7 and 45.7 cm. from the start of the main stem control a Standard Electric 1/100 sec. timer which

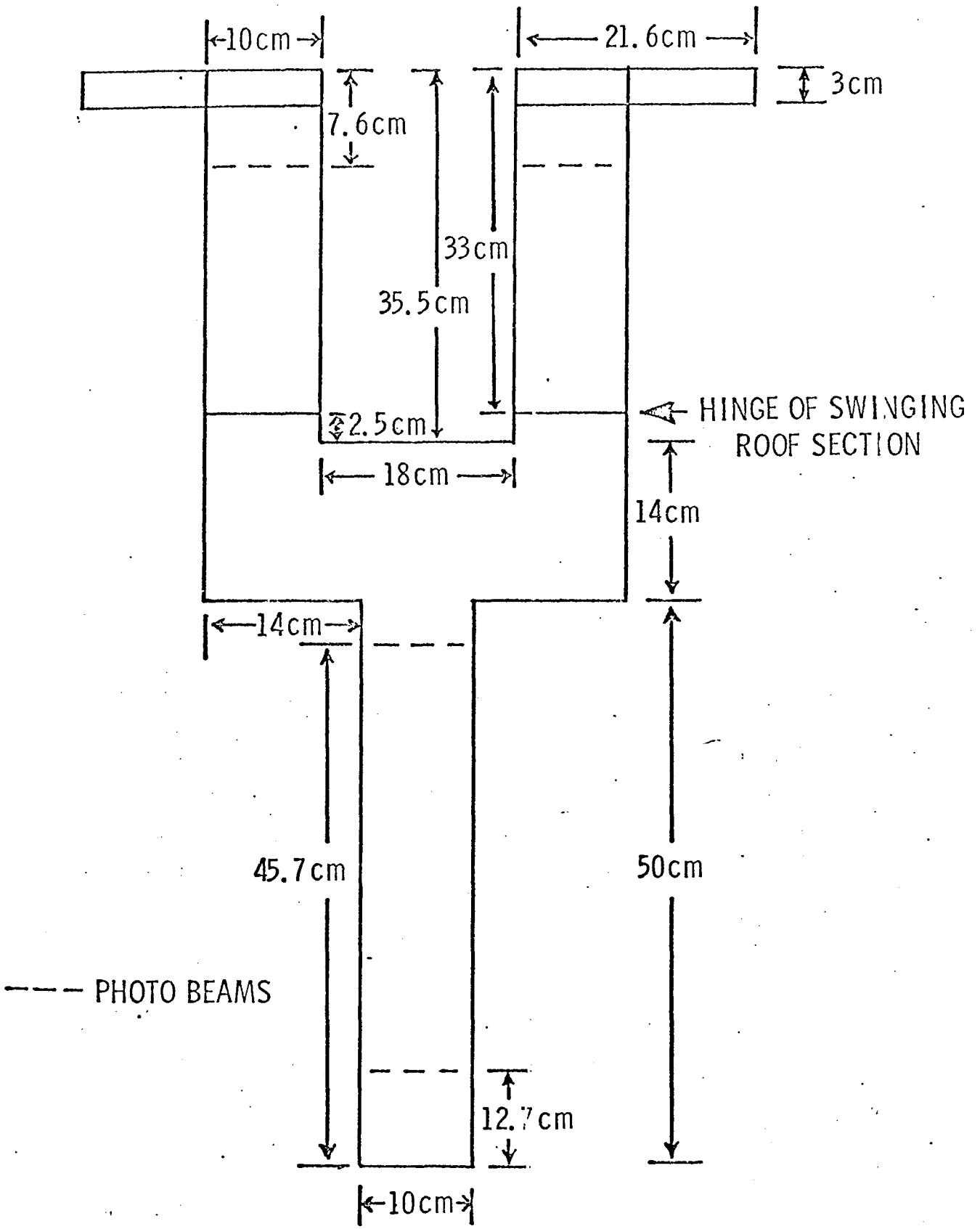

FIGURE 1

SCHEMATIC OF THE U-MAZE

records the latency of each response. Another photoelectric unit is mounted 7.6 cm. from the rear of each goal box. When S interrupts one of these goal box photobeams, an electric brake is released which allows a counter weighted 13.3 cm. section of the plexiglas roof to swing down behind S, forming a 33 cm. long goal box. The brake is then automatically reactivated, locking the door in place and confining S to the goal area.

Rotron Whisper Fans mounted below each goal box continually move the air throughout the entire maze, pulling air into the maze through two slots at the beginning of the main stem just below the hardware cloth floor and out of the maze just in front of the food trays. Noyes pellets were liberally distributed on the table below the maze floor throughout the goal and choice areas to mask any odors from the food trays.

Procedure

After determination of ad libitum body weights, all Ss were placed on 23 hr. food deprivation for the duration of the experiment. The Ss were fed Wayne Mouse Breeder Blox for one hour each day at about the same time of day that they were later observed in the U-maze. The number of food blox eaten by each S was recorded each day, and body weights were recorded every second day until acquisition training was begun and every third day thereafter. Deprivation was carried out for 62 days before any experimental treatments were given. The abnormally long deprivation period was necessitated by late delivery of the P-CPA by the supplier. After 62 days of deprivation, Ss were randomly divided

into three groups. The drug group (group D, n = 22) received at each feeding session 25 cc. of a solution containing 50 mg. of dl para-chlorophenylalanine (P-CPA) purchased from Nutritional Biochemical Corporation, 420 mg. of Imperial table sugar, and 0.2 cc. of Vidalin vitamins, manufactured by Abbot Laboratories, all dissolved in water. The P-CPA dosage amounted to approximately 100 mg/kg of ad libitum body weight per day.

The drug control group rats (group C, n = 20) received the same solution as group D Ss except that it contained no P-CPA. A yoked control group (group Y, n = 10) received the same control solution as group C Ss. Groups Y and C differed only with respect to the behavioral situation they encountered. Six additional Ss were treated like group C rats. These six rats were not used in the behavioral test but supplied the brain tissue required to calibrate the spectrofluorometer for the later serotonin measurements. These six rats will be referred to as "standards."

When any rat finished its 25 cc. of solution for that day it was given ad libitum water until the next feeding. The drinking bottles were checked three times per day to insure that no S was without water for more than eight hours. An animal received fresh drinking water only if it drank its 25 cc. of solution for that day. If any rat did not drink its 25 cc. of solution, at the next feeding session the remaining solution was discarded and a fresh solution was put in that rat's drinking bottle. During the first 10 days of drug treatment, the sugar concentration in the drug and control solutions was varied to determine that sugar concentration producing maximum drug solution intake.

Twenty-four hr. after the 28th drug treatment, six drug rats, six control treated rats, and two standards were decapitated. Their brains were extracted and frozen for later assay for 5HT.

The remaining Ss were placed individually each day for four days in one of the two goal boxes of the U-maze until each S ate 97 mg. Noyes Pellets for one minute without interruption. Each S was fed twice in each of the two goal boxes in alternating order. Following the four days of rewarded goal placements, acquisition training was begun.

Throughout acquisition, three trials per day were given. The first trial of the day for each rat was rewarded with 40 sec. in the goal box with the bottom of the food tray covered with 97 mg. Noyes pellets. On the two subsequent trials, each S in groups D and C was rewarded with 40 sec. in the baited goal box only if that S chose the arm of the maze opposite the arm it chose on the previous trial. On trials two or three, if a rat chose the same maze arm as it chose on the previous trial, it was confined to the goal box of the chosen arm for 40 sec. with the food tray in that goal box empty.

The 10 group Y Ss were yoked to 10 randomly selected Ss in group C. The group Y Ss were always rewarded on trial one but were rewarded on trial two or trial three only if their counterparts in group C were rewarded for alternating responses on those trials. Group Y was included in this design to provide a baseline level of alternation against which to compare the performance of groups D and C. Group Y rats received the same number of rewards in the same sequences as 10 of the Ss in group C. The only difference is that the rewards were contingent on behavior in

group C, but not in group Y. In summary, group D rats received P-CPA and had reward contingent on their alternation behavior. Group C rats received a control solution and contingent reward. Group Y Ss received the control solution and encountered rewards and nonrewards which were noncontingent on their own maze choices.

Acquisition training was given for 30 days with an intertrial interval (ITI) of 30 minutes. Throughout acquisition, latency, the direction of choice, and whether that choice was an alternation or not was recorded for each trial. Latency is defined as the time elapsed between the breaking of the first photobeam, when S was placed in the start arm, and the breaking of the second photobeam. If an S broke the second photobeam and then retraced into the start arm, the latency recorded for that trial was the time elapsed until the S first broke the second photobeam.

During the first five days of training, if any S failed to reach the second photobeam within five minutes, it was urged manually to the choice point. The maximum latency recorded for any trial was 60 sec. throughout the experiment. Once S reached the choice point, it was allowed five minutes in which to enter one of the goal arms. If an S did not enter a goal arm within this period of time, it was urged manually into the rewarded goal arm and allowed to remain until it ate for 1 min. On day six and thereafter, Ss were given only 60 sec. in which to reach the second photobeam before being urged into the choice point, and no S was forced to choose a goal arm after day six.

After acquisition training on day seven and thereafter, all Ss were visually examined for signs of drug induced malaise and any

symptoms observed were recorded. The symptoms observed were excessive weight loss with balding, or diarrhea stains. Ss that showed any of the symptoms received daily injections of the antibiotic "Bactrovet" until the symptoms were no longer evident. Nine of the P-CPA treated Ss required antibiotic treatments throughout acquisition. Three other P-CPA Ss required antibiotics only on acquisition days 12-30.

At the end of 30 days of training, the alternation data were grouped into six blocks of five days each. The total number of alternations made by each S for each block was calculated. These sums were then analyzed with a repeated measures analysis of variance. Because there was no superiority in alternation performance of the reward contingent Ss relative to the group Y Ss with the 30 min. ITI (acquisition phase 1), the ITI was reduced to 30 sec. and acquisition training was continued for an additional 20 days with the reduced ITI (acquisition phase 2).

The drugged Ss continued to receive regular doses of P-CPA for six days following completion of acquisition phase 2 training. Twenty-four hr. after the last P-CPA administration, at the time the Ss would usually be given a fresh drug solution, the drugged Ss, 10 randomly selected group C or Y Ss, and 4 standard rats were decapitated. Their brains were removed and frozen for later serotonin assay.

Brain Serotonin Assay

The brain assay involves extracting the brain amines from tissue by the method of Wiegland and Perry (1961). The amines are extracted into butanol from a salt-saturated aqueous phase at pH 2.0. They are

then returned to 0.01 N HCL by shaking the butanol with an excess of heptane and the aqueous phase.

Serotonin is measured directly on the aqueous phase following the addition of HCL, which shifts the fluorescence from the 350 mμ wavelength to 540 mμ. The improved method of Wise (1967) was used for reading the fluorescence at this wavelength. This method employs a modified #5 slit arrangement in which a Kodak Polar Screen is placed in the #4 position, thereby removing the second order scatter at the 600 mμ wavelength.

CHAPTER IV

RESULTS

Serotonin Depletion

The brain serotonin analyses performed on drug and control Ss before and after acquisition training yielded the number of micrograms of serotonin per gram of brain tissue for each rat. Analyses were performed on the brains of 16 control treated Ss and 28 P-CPA treated Ss. Control brain 5-HT values, whether obtained before (6 Ss), or after acquisition (10 Ss) were averaged to obtain a mean control brain 5HT concentration per gram of brain tissue as shown in Table 1. This mean control brain 5HT concentration (Control $\overline{X}$) was then used to compute the percent difference (%D) of each S's 5-HT concentration (X) from the mean control concentration according to the formula

$$\%D = \frac{X - \text{Control } \overline{X}}{\text{Control } \overline{X}}$$

Results of the 5HT analyses of control brains are shown in Table 1. Serotonin levels of P-CPA treated Ss examined after 28 days of drug treatment (brains examined before acquisition), or of P-CPA treated Ss whose brains were examined after 88 days of drug treatment (after acquisition) are presented in Tables 2A and 2B respectively. P-CPA produced a dramatic decrease in 5HT levels after 28 days of drug treatment and 5HT levels remained low after 60 additional days of drug administration.

TABLE 1

CONTROL BRAIN SEROTONIN CONCENTRATIONS AND THE

PERCENT DIFFERENCE FROM THE MEAN OF THE CONTROL VALUES

Control Ss Subject Number	Serotonin in µg of 5HT per gm. of Tissue		Percent Difference from Control $\bar{X}$
1	.62		+19%
2	.56		+ 8%
3	.52		0%
4	.51		- 2%
5	.54		+ 4%
6	.63		+21%
7	.63		+21%
8	.72	Control Brain $\bar{X}$ = .52	+38%
9	.57		+10%
10	.56		+ 8%
11	.66		+27%
12	.50		- 4%
13	.59		+13%
14	.53		+ 1%
15	.03		-94%
16	.09		-83%

TABLE 2A

BRAIN SEROTONIN CONCENTRATIONS OF P-CPA TREATED RATS AFTER 28 DAILY P-CPA TREATMENTS AND THE PERCENT DIFFERENCE FROM THE MEAN CONTROL BRAIN CONCENTRATION

Subject Number	Serotonin in µg of 5HT per gm. of Tissue	Percent Difference from Control $\bar{X}$
1	.09	-83%
2	.08	-85%
3	.08	-85%
4	.05	-90%
5	.08	-85%
6	.05	-90%

TABLE 2B

BRAIN SEROTONIN CONCENTRATIONS OF P-CPA TREATED RATS AFTER 88 DAILY P-CPA TREATMENTS AND THE PERCENT DIFFERENCE FROM THE MEAN CONTROL BRAIN CONCENTRATION

Subject Number	Serotonin in µg of 5HT per gm. of Tissue	Percent Difference from Control $\bar{X}$
1	.05	-90%
2	.04	-92%
3	.02	-96%
4	.02	-96%
5	.05	-90%
6	.05	-90%
7	.05	-90%
8	.05	-90%
9	.05	-90%
10	.05	-90%
11	.06	-88%
12	.05	-90%
13	.01	-98%
14	.04	-92%
15	.02	-96%
16	.03	-94%
17	.02	-96%
18	.04	-92%

TABLE 2B, cont.

Subject Number	Serotonin in ug of 5HT per gm. of Tissue	Percent Difference from Control $\bar{X}$
19	.04	-92%
20	.04	-92%
21	.58	+12%
22	.56	+ 8%

The mysterious fact that two of the P-CPA treated Ss (#21,22) showed no brain 5HT depletion and two of the nondrugged control Ss (#15, 16) showed extreme 5HT depletion must be considered. Great care was taken to insure that there were no errors during the drug or control solution administration. The observed anomalies are probably due to errors either in the labeling of the four brains after they were removed from the donors or to labeling errors when the brain 5HT concentrations were recorded. Behavioral data from these four Ss were retained in all subsequent analyses. All of the other drug or control brain 5HT concentrations are perfectly consistent with the appropriate drug or control values obtained by Koe and Weissman (1968) with P-CPA under similar conditions, doses, and drug durations.

Body Weights

Body weights of P-CPA treated and of nondrugged Ss were recorded every second day during the eight days immediately prior to the beginning of drug treatment (pre-drug period; eight days) and during the drug treatment period preceding acquisition (pre-acquisition drug treatment period; 32 days). Group mean body weights of the P-CPA treated and nondrugged Ss from these two periods are plotted in Figure 2. The nondrugged and drugged groups include only Ss that received acquisition training (either contingent reward or yoked reward). The P-CPA treated Ss lost weight after drug treatment was initiated. Drug group mean body weights then stabilized before the end of the pre-acquisition period.

The group means of the body weights obtained every third day during acquisition phases 1 and 2 for the P-CPA treated and nondrugged Ss are

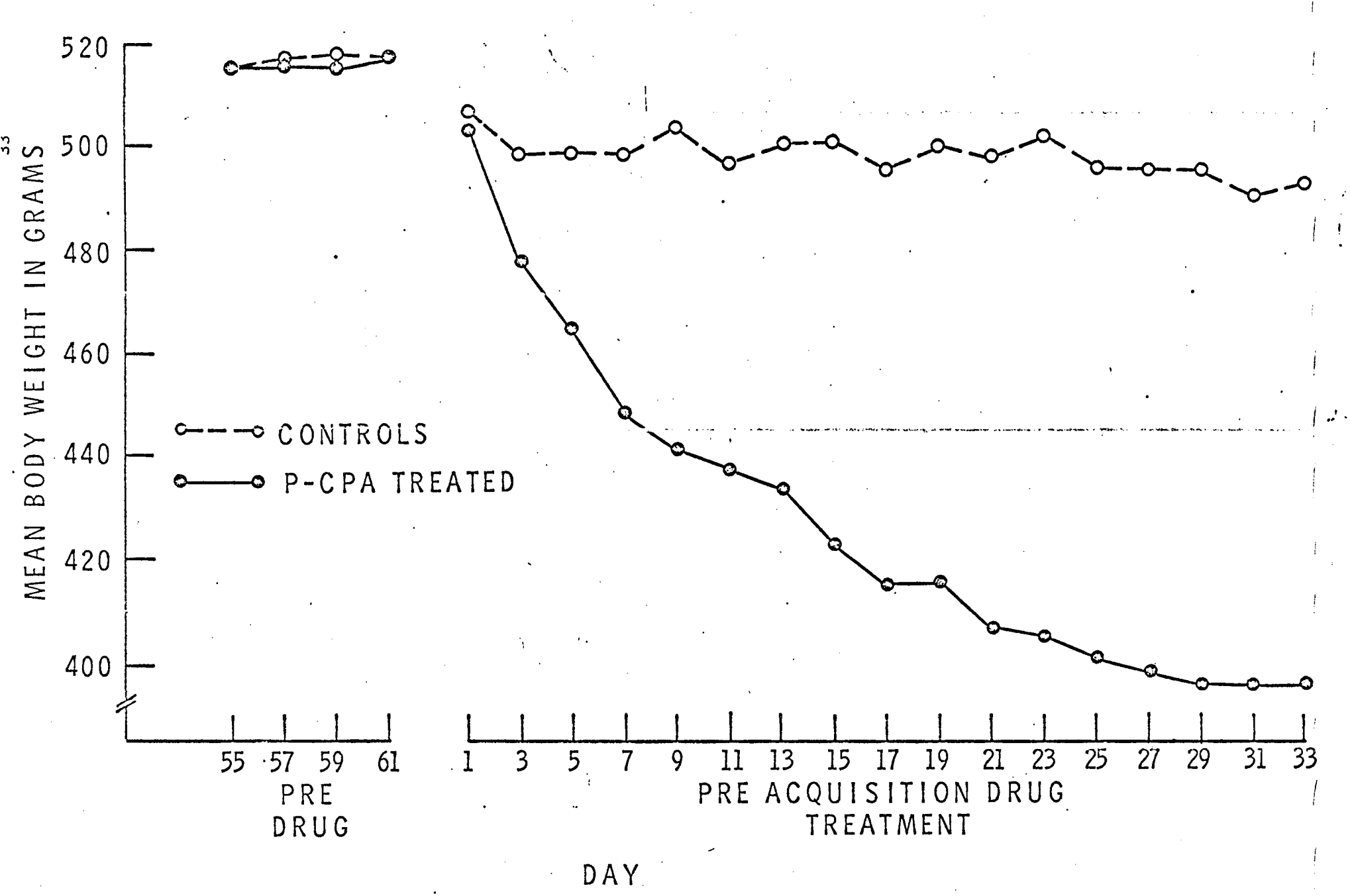

FIGURE 2

GROUP MEAN BODY WEIGHTS OF THE P-CPA TREATED AND NONDRUGGED CONTROL Ss (CONTINGENT AND NONCONTINGENT REWARD Ss) DURING THE PRE-DRUG AND PRE-ACQUISITION

plotted in Figure 3. Drug group mean body weights did not decrease further during acquisition but remained stable at about 400 grams.

Food Intake

The number of Wayne Mouse Breeder Blox consumed during each daily one hour feeding session was recorded for each S and these scores were grouped into blocks for analysis. The pre-drug and pre-acquisition drug treatment period food scores were grouped into four day blocks. Food consumption scores from acquisition phases 1 and 2 were grouped into five day blocks for analysis. Because the average size of the mouse food blox changed several times during the experiment, any main effect of the trial blocks factor in a repeated measures analysis of variance of food consumption scores may not actually reflect differential food consumption as a function of blocks but may merely reflect a change in the average size of the food blox. However, the size of the food blox did not vary consistently between groups on any day; therefore, a significant main effect on food consumption of the between subjects factor "Groups" would be interpretable as a difference in food consumption due to differential treatment of the groups.

The effects of P-CPA treatment on food consumption were evaluated by comparing food blox intake scores of the drugged and nondrugged Ss (only those that received acquisition training) with a repeated measures analysis of variance. The pre-drug period food scores are plotted in Figure 4. The analysis of these scores indicated no effect of the dummy variable "Drug Condition" (DC: $F = .02$, $df = 1,50$; $p > .10$). Also shown in Figure 4 are the group mean food scores of the P-CPA

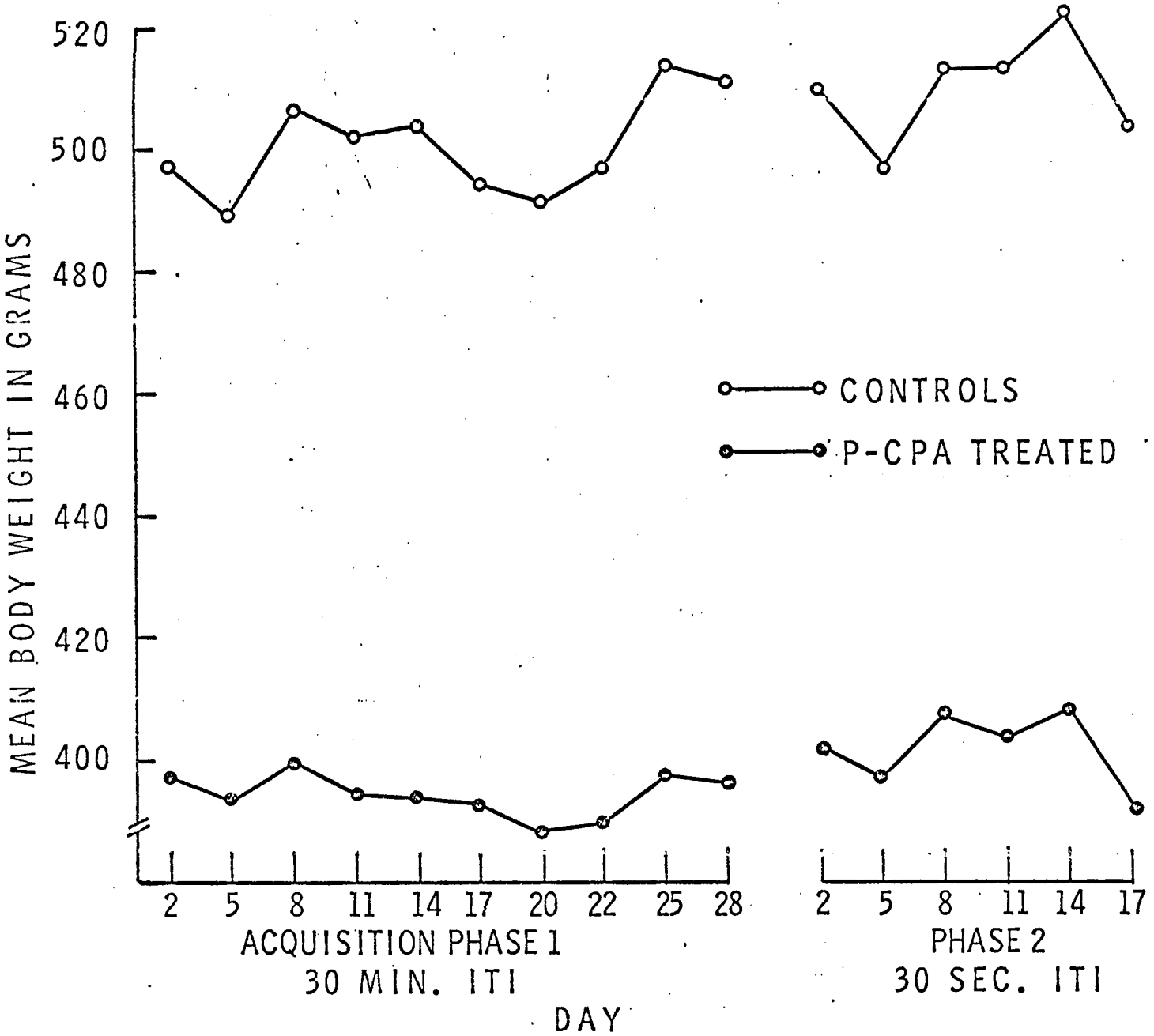

FIGURE 3

GROUP MEAN BODY WEIGHTS OF THE P-CPA TREATED AND NONDRUGGED CONTROL Ss (CONTINGENT AND NONCONTINGENT REWARD Ss) DURING ACQUISITION PHASES 1 AND 2

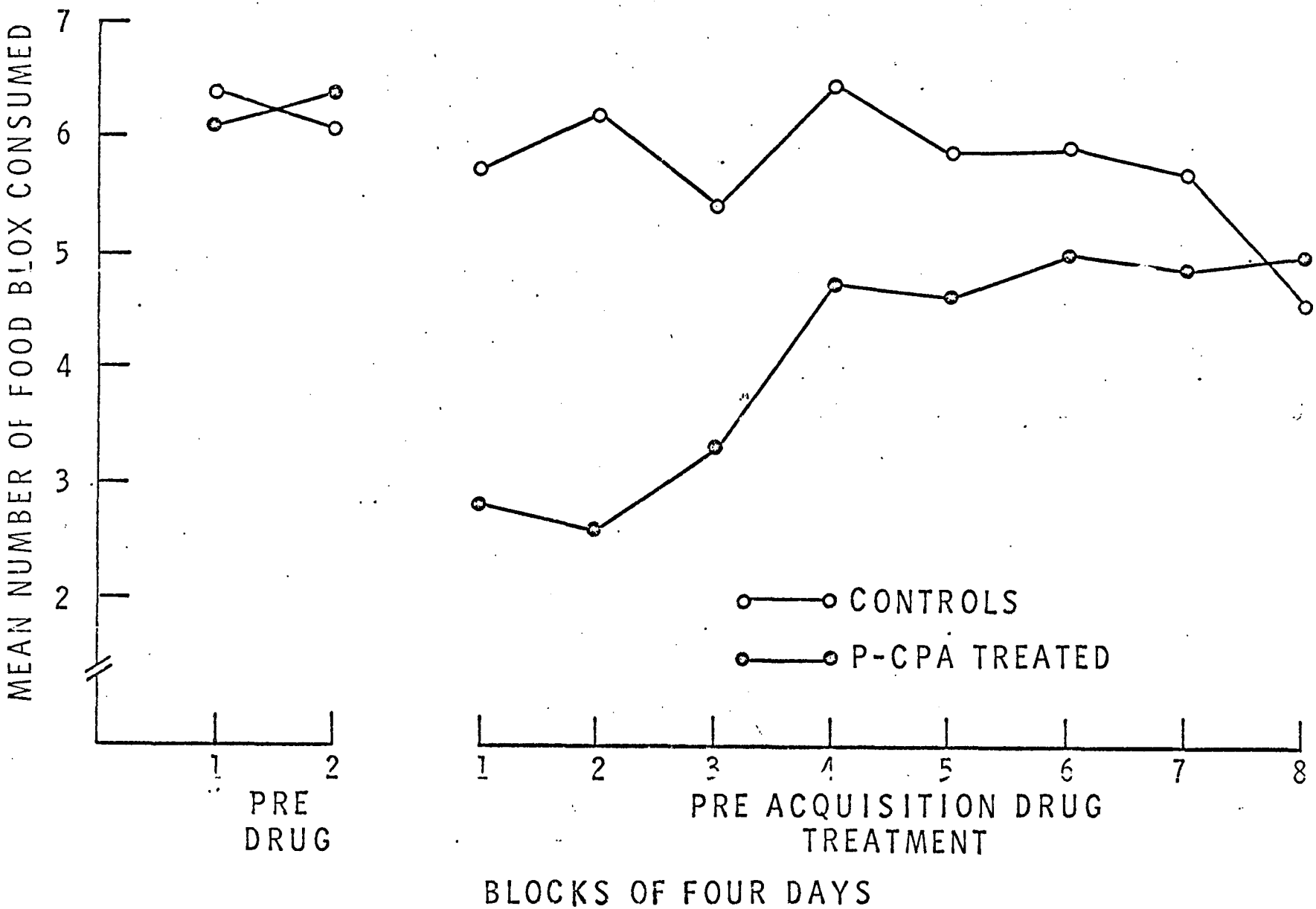

FIGURE 4

GROUP MEAN FOOD CONSUMPTION SCORES OF THE P-CPA TREATED AND NONDRUGGED CONTROL Ss (CONTINGENT AND NONCONTINGENT REWARD Ss) DURING THE PRE-DRUG AND THE PRE-ACQUISITION DRUG TREATMENT PERIOD IN FOUR DAY BLOCKS

treated and nondrugged Ss obtained during the pre-acquisition drug treatment period when the P-CPA was actually being administered. Analysis of variance of the food scores from the pre-acquisition drug treatment period revealed a highly significant main effect of the drug condition (DC: $F = 41.07$, $df = 1,50$; $p < .01$). The entire analysis is summarized in Table 3. The DC main effect is due to the immediate and pronounced decrease in food consumption when P-CPA treatment was begun. The significant interaction of Drug Condition X Blocks (DC X B: $F = 297.92$, $df = 7,350$; $p < .01$) reflects the subsequent rise of the food consumption scores in the P-CPA treated group as drug treatment continued (see Figure 4).

Group mean food intake scores of the P-CPA treated and nondrugged Ss that were recorded during acquisition phases 1 and 2 are plotted in Figure 5 in five day blocks. An analysis of variance of the phase 1 food intake data is summarized in Table 4. The main effect of the drug condition is again highly significant (DC: $F = 36.94$, $df = 1.50$; $p < .01$) and again reflects the depression of food intake by P-CPA treatment. The DC main effect was also significant when acquisition phase 2 food scores were examined with an analysis of variance (DC: $F = 26.03$, $df = 1,50$; $p < .01$). The depression of food intake by P-CPA occurred as long as drug treatment was continued.

Because nine of the P-CPA treated Ss became ill enough to require antibiotic treatment, it is possible that the depression of food consumption by P-CPA was due to a general malaise induced by the P-CPA, or that the depression of food consumption was caused by the antibiotics

TABLE 3

ANALYSIS OF VARIANCE OF PRE-ACQUISITION DRUG TREATMENT PERIOD FOOD CONSUMPTION SCORES IN FOUR DAY BLOCKS COMPARING THE P-CPA TREATED AND THE NONDRUGGED Ss

Source	df	MS	F
Between subjects			
DC (drug condition)	1	275.20	41.07**
SwG (subj. w. groups)	50	6.70	
Within subjects			
B (blocks)	7	12.31	102.58**
DC X B	7	35.75	297.92**
B X DC X SwG	350	.12	

** $p < .01$

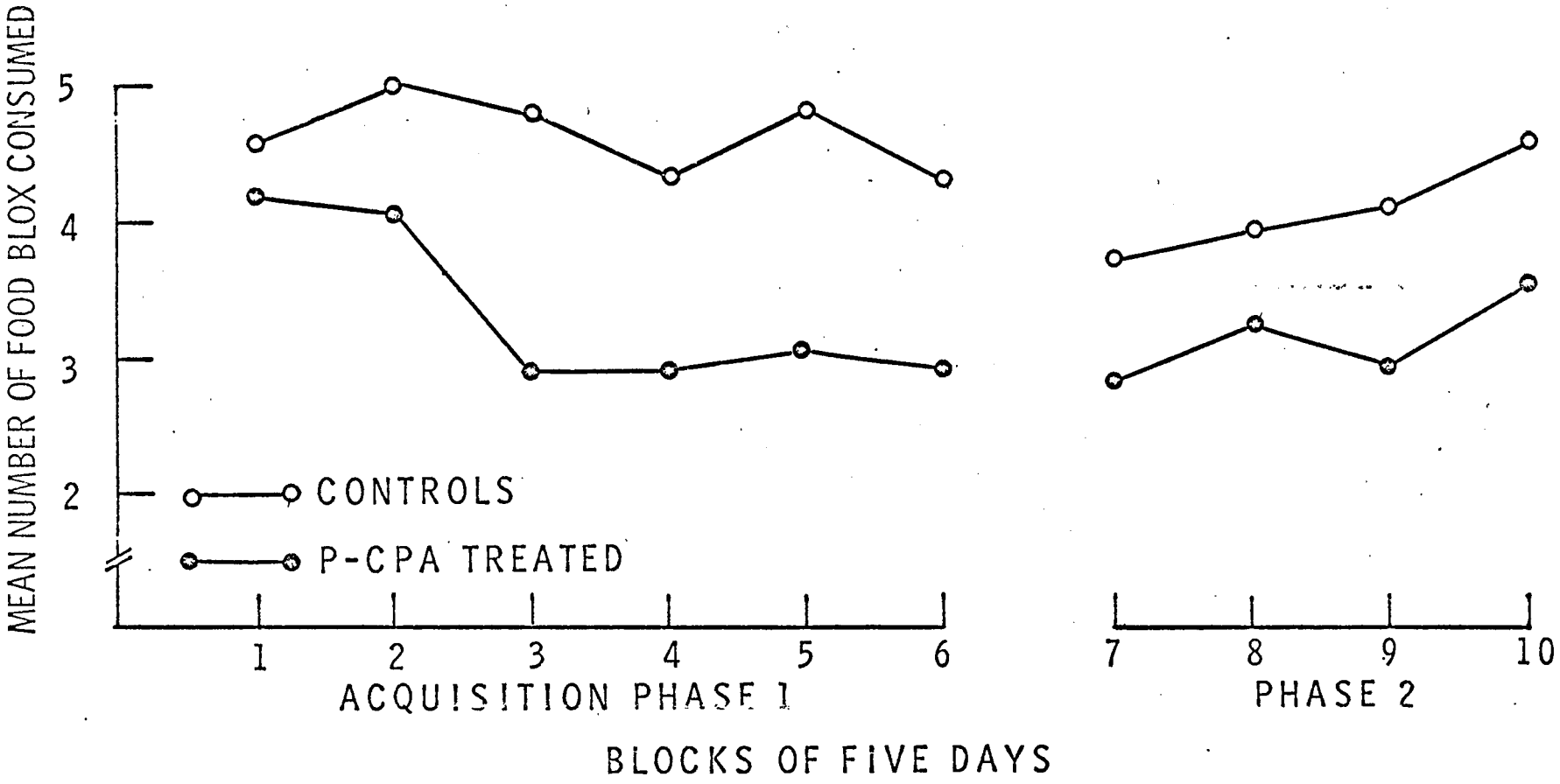

FIGURE 5

GROUP MEAN FOOD CONSUMPTION SCORES OF THE P-CPA TREATED AND NONDRUGGED CONTROL Ss (CONTINGENT AND NONCONTINGENT REWARD Ss) DURING ACQUISITION PHASES 1 AND 2 IN FIVE DAY BLOCKS

TABLE 4

ANALYSIS OF VARIANCE OF FOOD CONSUMPTION

SCORES FROM ACQUISITION PHASE 1 IN FIVE DAY BLOCKS

COMPARING THE P-CPA TREATED AND NONDRUGGED Ss

Source	df	MS	F
Between subjects			
DC (drug condition)	1	121.17	36.94**
SwG (subj. w. groups)	50	3.28	
Within subjects			
B (blocks)	5	7.25	15.10**
B X DC	5	4.10	8.54**
B X DC X SwG			

** $p < .01$

given the nine ill Ss and was only indirectly related to P-CPA treatment. Both possibilities are unlikely. Maximum depression of food intake occurred immediately after initiation of P-CPA treatment and before antibiotic treatments were begun. Also, an analysis of food intake during acquisition phase 2 comparing P-CPA Ss that required antibiotic treatments to those P-CPA Ss that did not require antibiotics showed no significant difference between the two P-CPA subgroups. This analysis is summarized in Table 5. The antibiotic main effect on the P-CPA subgroups did not reach the .05 level of significance (A: $F = 3.21$, $df = 1,20$; $p > .05$). Figure 6 reveals that even the 13 P-CPA treated Ss that showed no obvious signs of malaise (balding, diahhrea, or excessive weight loss) and did not receive antibiotics ate less than the control Ss not treated with P-CPA. An analysis of variance of phase 2 food consumption scores comparing only the 13 P-CPA treated Ss that did not require antibiotics to the nondrugged rats yielded a significant drug main effect (D: $F = 13.45$, $df = 1,41$; $p < .01$). These results indicate that the P-CPA treatment depresses food intake, even in the absence of obvious P-CPA induced malaise and antibiotic therapy.

Acquisition of the Runway Response - Decreases in Latencies

All latency scores were transformed to Log 10X for analysis. To examine the effects of the P-CPA on acquisition of rapid running in the maze, the first trial latencies of groups D and C from each day were grouped into five day blocks for analysis. The first daily trial was chosen for the unit of analysis because all Ss received reward on trial one, independent of alternation proficiency.

TABLE 5

ANALYSIS OF VARIANCE OF ACQUISITION PHASE 2 FOOD CONSUMPTION SCORES IN FIVE DAY BLOCKS COMPARING THE P-CPA TREATED Ss THAT REQUIRED ANTIBIOTICS TO THOSE P-CPA TREATED Ss THAT DID NOT REQUIRE ANTIBIOTICS

Source	df	MS	F
Between subjects			
A (antibiotic)	1	6.29	3.21
SwG (subj. w. groups)	20	1.96	
Within subjects			
B (blocks)	3	2.65	8.83**
A X B	3	.06	.20
A X B X SwG	60	.30	

** $p < .01$

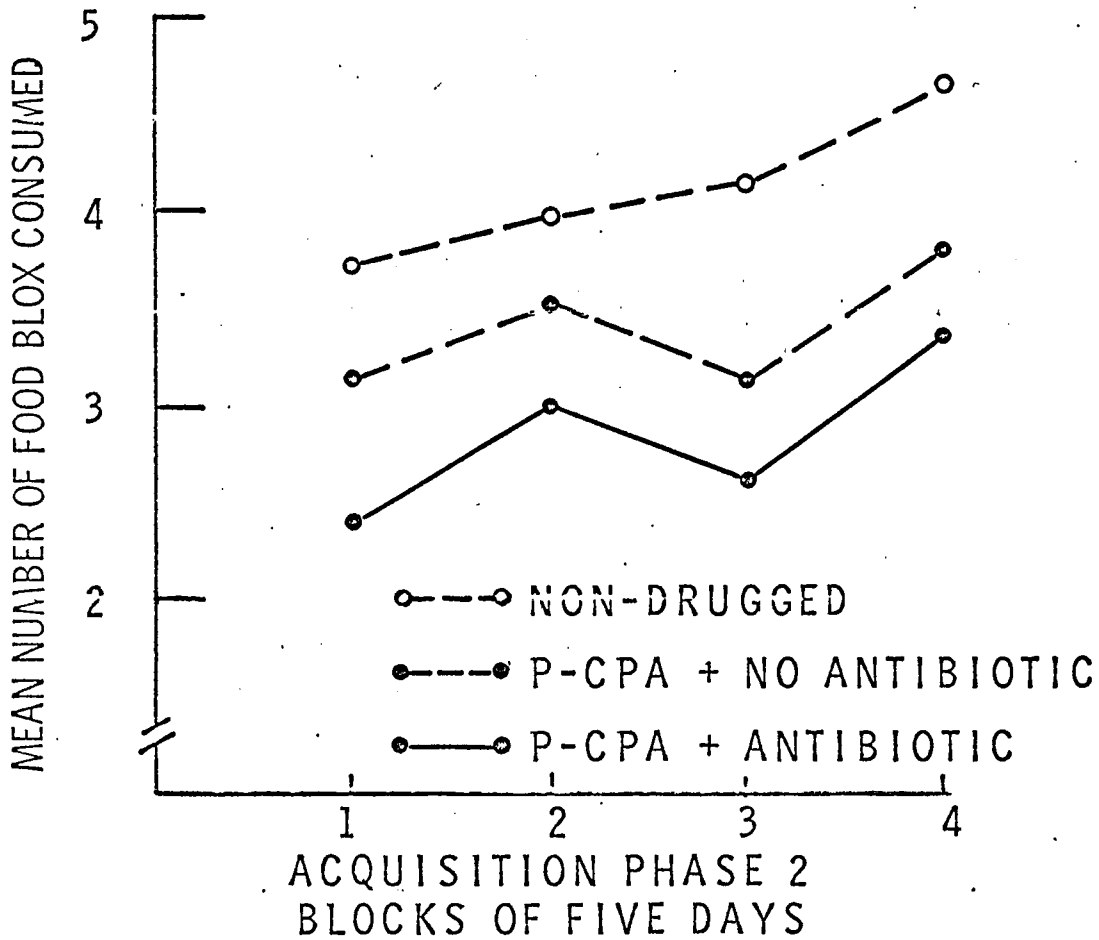

FIGURE 6

GROUP MEANS OF THE FOOD CONSUMPTION SCORES FOR ACQUISITION PHASE 2 IN FIVE DAY BLOCKS FOR THE NONDRUGGED CONTROL Ss (CONTINGENT AND NONCONTINGENT REWARD Ss), THE P-CPA TREATED Ss THAT REQUIRED ANTIBIOTIC THERAPY, AND THE P-CPA TREATED Ss THAT DID NOT REQUIRE ANTIBIOTIC THERAPY

Trial one mean latencies of the drug and control groups in five day blocks are plotted in Figure 7. Latencies of the yoked group were excluded to simplify analysis. The decrease in first trial latencies during early phase 1 training shows the negative acceleration typical of learning curves of running times in appetitive reward situations. An analysis of variance of the first trial latencies from acquisition phase 1 is summarized in Table 6. While there was no significant main effect of the P-CPA drug condition (DC: $F = 2.47$, $df = 1.40$; $p > .10$) on first trial latencies, there was a significant interaction of Drug Condition X Blocks (DC X B: $F = 2.67$, $df = 5,200$; $p < .05$). The interaction indicates that the rate of decrease of running times differed between P-CPA treated and nondrugged controls. With reference to Figure 7, it appears that the interaction is caused by the fact that the drugged Ss ran much slower than the nondrugged Ss on block one of training, there being no difference in running times between the groups by block three.

Trial one group mean latencies from acquisition phase 2 in blocks of five days are also plotted in Figure 7. Inspection of Figure 7 reveals no difference in first trial running times between drug and control Ss. Analysis of variance of the phase 2 trial one latencies comparing the drug and control groups yielded all F ratios less than unity. These results indicate that while P-CPA treatment may have facilitated rate of learning in early acquisition, the drug did not affect asymptotic running times.

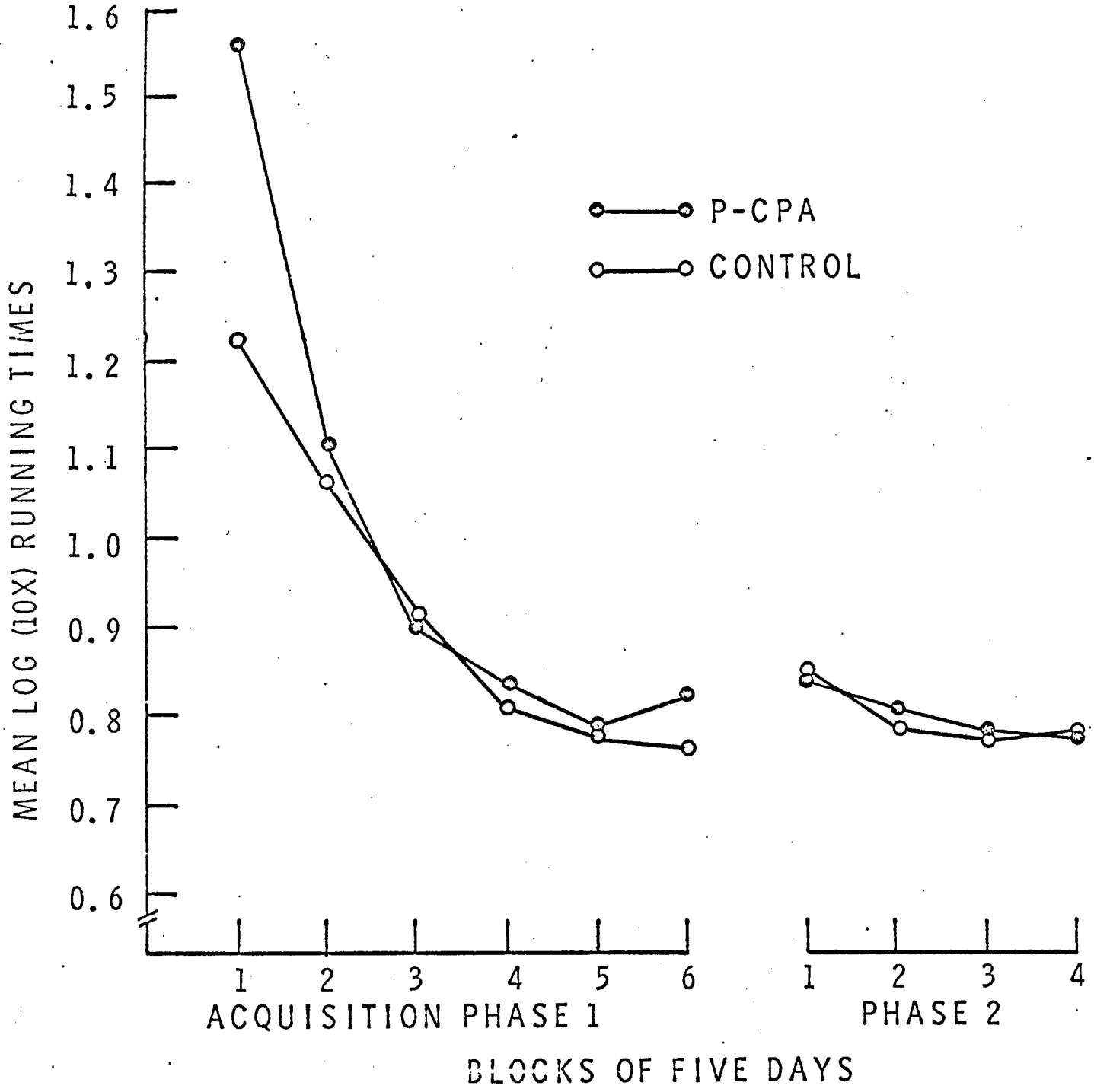

FIGURE 7

TRIAL 1 MEAN LATENCIES OF THE P-CPA TREATED GROUP AND THE CONTROL

TABLE 6

ANALYSIS OF VARIANCE OF FIRST TRIAL LATENCIES

IN FIVE DAY BLOCKS FROM ACQUISITION PHASE 1

COMPARING THE P-CPA TREATED TO THE CONTROL GROUP

Source	df	MS	F
Between subjects			
DC (drug condition)	1	.42	2.47
SwG (subj. w. groups)	40	.17	
Within subjects			
B (blocks)	5	2.48	41.33**
DC X B	5	.16	2.67*
DC X B X SwG	200	.06	

** $p < .01$

* $p < .05$

Alternation Behavior

The alternation scores were grouped into five day blocks by computing each S's total number of alternations for each five day period. There were only two possible alternations per day for each rat, or a maximum of 10 alternations per block for each S. The experimental design of this study involves both random and matched sampling methods. The drug group Ss were randomly selected with respect to the yoke and control Ss. But the yoked subjects must be treated as matched to their 10 control group counterparts when alternation scores of the two groups are considered. Because both matched and random sampling were employed in this study, overall analyses of variance of the alternation data assuming a particular type of sampling were not appropriate. Instead, drug effects during acquisition phases 1 and 2 had to be evaluated by comparing the drug group with one of the nondrug groups, using an analysis assuming random sampling. Evaluation of the reward contingency effects required the comparison of the yoked rats to their 10 reward contingent counterparts with an analysis assuming matched subject sampling.

Phase 1 alternation - 30 minute ITI. Group mean alternation scores from acquisition phases 1 and 2 for the drug, control, and yoked groups in five day blocks are plotted in Figure 8. An examination of the effect of the reward contingency on phase 1 alternation was performed with a matched groups analysis of variance of the blocked alternation scores from phase 1. The analysis of variance compared the alternation behavior of the 10 yoked Ss to that of their reward contingent counterparts

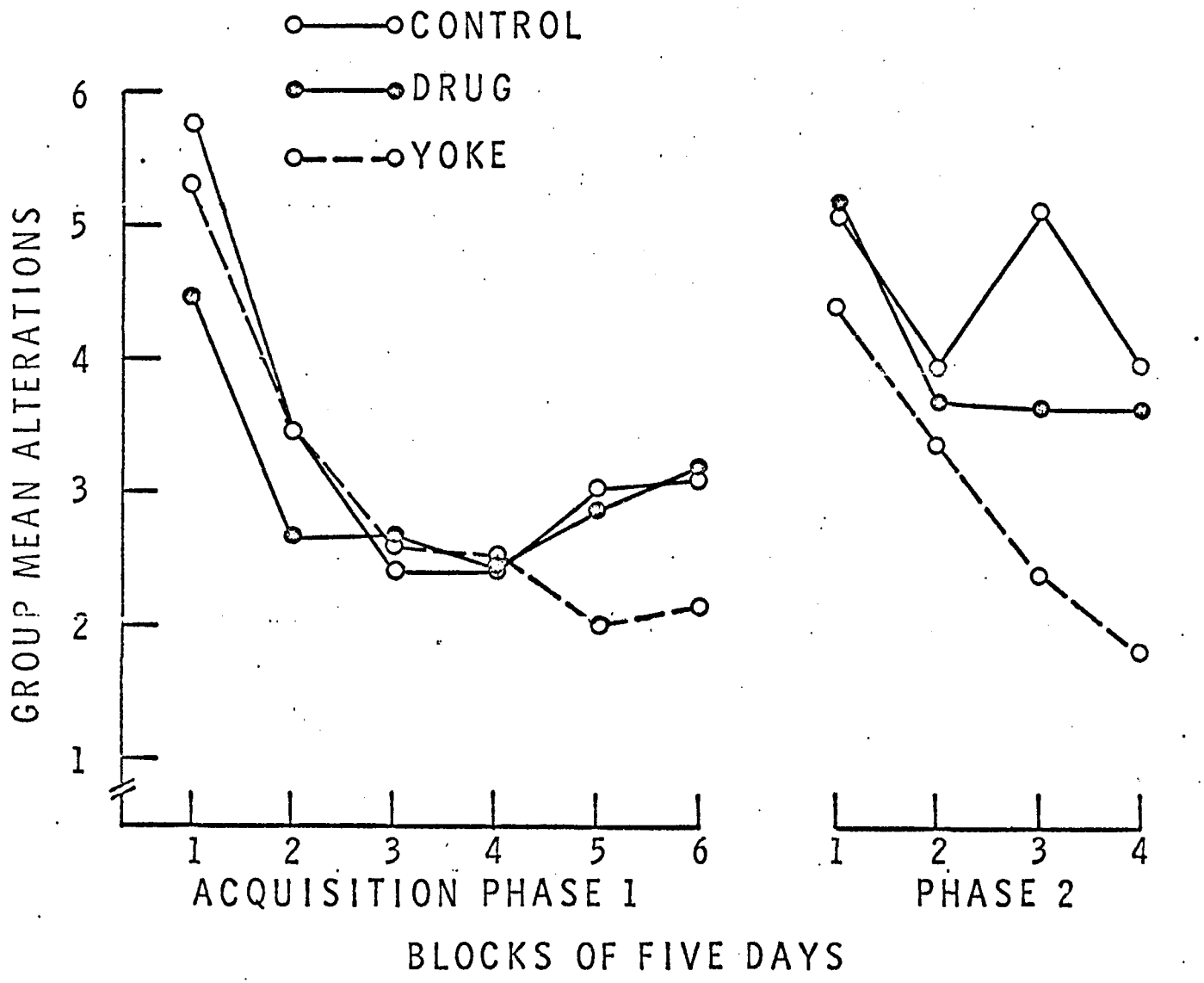

FIGURE 8

GROUP MEAN ALTERNATION SCORES OF THE P-CPA TREATED, CONTROL, AND YOKE GROUPS FOR ACQUISITION PHASES 1 AND 2 IN FIVE DAY BLOCKS

in the control group. The means of these two groups are plotted in Figure 9. The results of the analysis are summarized in Table 7. The main effect of the reward contingency (RC: $F < 1$) did not approach significance. An analysis of variance on only the data from blocks 5 and 6 of phase 1 also yielded no significant main effect of the reward contingency on alternation performance (RC: $F < 1$).

Phase 2 alternation - 30 second ITI. The failure to find an effect of the reward contingency on alternation performance during phase 1 prompted the reduction of the ITI from 30 minutes to 30 seconds. Group mean alternation scores from the 30 sec. ITI phase of the experiment (phase 2) are plotted in Figure 8. There was an apparent immediate facilitation of alternation performance when the ITI was shortened. To determine if the facilitation was reliable and if there was an effect of reward contingency on the amount of facilitation that occurred, the alternation scores on the last block of phase 1 and on the first block of phase 2 were compared for the 10 yoked Ss and for their 10 matched group C partners with an analysis of variance. A significant main effect of Blocks was obtained (B: $F = 27.56$; $df = 1,9$; $p < .01$), indicating that the improvement in alternation performance when the ITI was shortened was reliable. The failure to find a Reward Contingency X Blocks interaction (RC X B: $F < 1.0$) suggests that the noncontingent reward group improved in alternation performance as much as the contingent reward Ss when the ITI was reduced.

The influence of the reward contingency, considering all of the phase 2 alternation scores, was evaluated with a matched groups analysis.

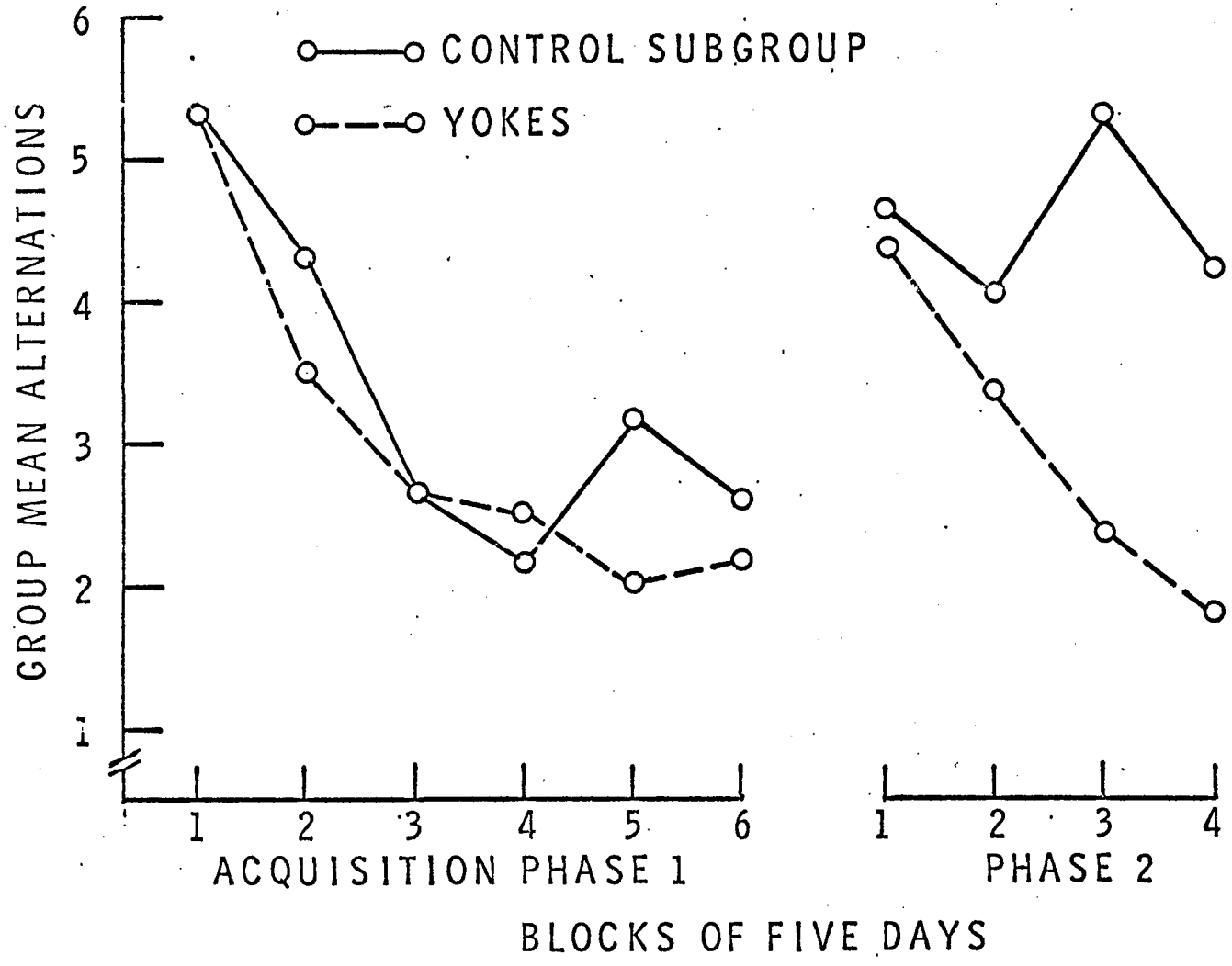

FIGURE 9

GROUP MEAN ALTERNATION SCORES OF THE 10 YOKE Ss AND THEIR 10 CONTINGENT REWARD PARTNERS (CONTROL SUBGROUP) FOR ACQUISITION PHASES 1 AND 2

TABLE 7

ANALYSIS OF VARIANCE OF ACQUISITION PHASE 1 ALTERNATION SCORES COMPARING THE 10 YOKED *Ss* (NONCONTINGENT REWARD) TO THEIR 10 CONTROL GROUP (CONTINGENT REWARD) PARTNERS

Source	df	MS	F
RC (reward contingency)	1	4.03	.47
B (blocks)	5	27.70	14.50 **
S (between subjects)	9	6.06	
B X RC	5	1.53	.41
B X S	45	1.91	
RC X S	9	8.63	
RC X B X S	45	3.75	

** $p < .01$

With this analysis of variance, the alternation scores of the 10 yoked Ss were compared to the scores of their 10 contingent reward counterparts in the control group for blocks one through four of acquisition phase 2 (Figure 9). The analysis is summarized in Table 8. While the main effect of reward contingency did not reach the .05 level of significance (RC: $F = 3.38$, $df = 1,9$; $.10 > p > .05$), the interaction of Reward Contingency X Blocks was significant (RC X B: $F = 3.55$, $df = 3,27$; $p < .05$). Examination of Figure 9 indicates that the RC X B interaction was caused by a decrease in alternation levels among the yoke Ss as phase 2 training progressed, while the contingent reward group continued at the elevated performance levels. An analysis of the reward contingency effect on phase 2, block four alternations yielded a significant effect of the reward contingency (RC: $F = 5.95$, $df = 1,9$; $p < .05$). These results indicate that the contingent reward group evidenced alternation levels above "spontaneous" alternation levels, and that this effect of reward contingency increased as phase 2 training progressed because of the decline in spontaneous alternation with continued training. In Figure 8 it appears that the drug group performed above spontaneous alternation levels. An analysis of variance comparing the drug and yoked Ss over blocks one through four of acquisition phase 2 was performed and is summarized in Table 9. The group main effect was not statistically significant when the non-directional F test was performed (G: $F = 3.86$, $df = 1,30$; $.10 > p > .05$). However, on the basis of previous findings, it was assumed that the P-CPA treated

TABLE 8

ANALYSIS OF VARIANCE OF ACQUISITION PHASE 2 ALTERNATION SCORES COMPARING THE 10 YOKED (NONCONTINGENT REWARD) Ss TO THEIR 10 CONTROL GROUP (CONTINGENT REWARD) PARTNERS

Source	df	MS	F
RC (reward contingency)	1	49.61	3.38
B (blocks)	3	7.58	4.62**
S (between subjects)	9	4.98	
RC X B	3	8.21	3.55*
B X S	27	1.64	
RC X S	9	14.64	
RC X B X S	27	2.31	

** $p < .01$

* $p < .05$

TABLE 9

ANALYSIS OF VARIANCE OF THE ALTERNATION SCORES FROM ACQUISITION PHASE 2 COMPARING THE DRUG AND YOKED GROUPS

Source	df	MS	F
Between subjects			
G (groups)	1	30.19	3.86
SwG (subj. w. groups)	30	7.82	
Within subjects			
B (blocks)	3	24.61	9.84**
B X G	3	2.44	.98
B X G X SwG	90	2.50	

** $p < .01$

Ss would perform at higher alternation levels than the "spontaneous" alternation levels of the yoked Ss. For this reason, a directional test of the drug-yoke comparison is appropriate. A directional test of the Group main effect was performed by converting the observed F value into a t value according to the relation $t = \sqrt{F}$ when two independent groups are compared. The value of t computed ($t = \sqrt{F} = 1.97$) exceeds the .05 significance level for a one-tailed t test with df = 30, indicating that the superiority of the drug Ss relative to the yoked Ss was reliable.

Next, the effect of the P-CPA treatment on reward contingent alternation performance was evaluated by comparing the alternation scores of the drug Ss to those of the 20 control Ss over acquisition phase 2 with an analysis of variance. The results of that analysis are presented in Table 10. Neither the main effect of drug treatment (DC: $F = 2.34$, df = 1,40; $p > .10$) nor the interaction of drug treatment with blocks (DC X B: $F = 2.03$, df = 3,120; $p > .10$) approached significance. The P-CPA treatment did not affect conditioned alternation responding during acquisition phase 2.

Finally, to determine if drug induced malaise affected the alternation behavior of P-CPA treated rats, phase 2 alternation scores of the 9 P-CPA Ss that showed signs of malaise were compared to the scores of the 13 P-CPA Ss that did not have symptoms of malaise. The mean phase 2 alternation scores of the two P-CPA subgroups are plotted in Figure 10. There appears to be no difference in the group mean alternation scores between the two P-CPA subgroups. An analysis of

TABLE 10

ANALYSIS OF VARIANCE OF ALTERNATION SCORES FROM ACQUISITION PHASE 2 COMPARING THE P-CPA TREATED Ss TO THE 20 CONTINGENT REWARD CONTROL Ss

Source	df	MS	F
Between subjects			
DC (drug condition)	1	11.65	2.34
SwG (subj. w. groups)	40	4.97	
Within subjects			
B (blocks)	3	18.50	7.03**
DC X B	3	5.33	2.03
DC X B X SwG	120	2.63	

** $p < .01$

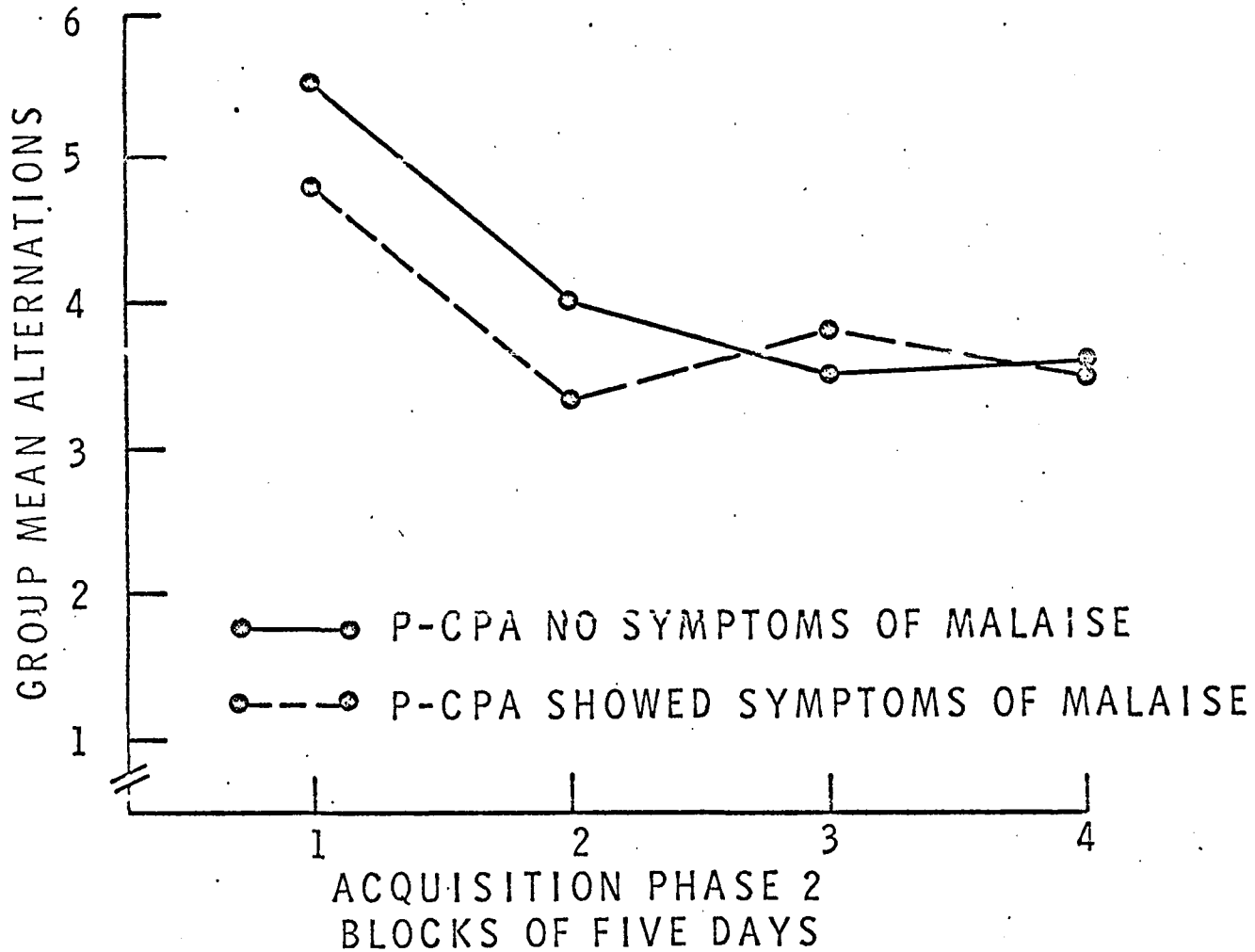

FIGURE 10

GROUP MEAN ALTERNATION SCORES FROM ACQUISITION PHASE 2 OF THE 9 P-CPA Ss THAT SHOWED PHYSICAL SYMPTOMS OF MALAISE AND OF THE 13 P-CPA Ss THAT SHOWED NO SYMPTOMS OF MALAISE

variance of the alternation scores from the two P-CPA subgroups yielded F ratios less than unity for both the main effect of groups and the interaction of groups by blocks. Phase 2 alternation behavior of the P-CPA treated Ss was independent of drug induced malaise.

CHAPTER V

DISCUSSION

Serotonin Depletion

The magnitude and permanence of brain serotonin depletion observed in this study following P-CPA treatment are quantitatively similar to the depletion findings of Koe and Weissman (1968) with the same drug. In the present study, treatment with P-CPA depleted brain serotonin by 82 to 91% of normal levels following 28 days of treatment, and 5HT levels remained low (85 to 98% below normal) following an additional 60 days of P-CPA treatment. The procedure of administering P-CPA in the drinking water offers a practical method of drug administration to adult rodents. Oral administration in the water supply avoids the difficulties of intubation of large rodents and also avoids the excessive tissue damage that results from repeated intraperitoneal injections.

Running Response Acquisition - Phase 1

Considering the drastic 5HT depletion obtained, it is surprising that there was no drug deficit during acquisition of rapid running in the maze stem, as indicated by decreasing first trial latencies on successive days of training. Instead, the drugged Ss may have evidenced faster acquisition of rapid running in the maze stem than the normal Ss.

Whether or not the observed interaction of Groups by Blocks during acquisition of rapid running reflects a different rate of learning of P-CPA treated versus normal rats is debatable. The interaction was caused by the fact that drugged Ss ran much slower than normals on the first block of trials, the groups running equally fast by trial block three. Situations of this type in which there are large differences between groups on initial performance levels, with the groups attaining equal terminal performance levels, often arise in extinction studies in the runway. The resultant interaction of Groups X Blocks when the data are analyzed with an analysis of variance is usually interpreted to mean that the groups were differentially resistant to extinction. Because of the similarities of the acquisition of rapid running to the extinction of rapid running (or the acquisition of slow running), the interpretation applied to the Groups X Blocks interaction during extinction is applied to these acquisition results. The Groups by Blocks interaction observed during acquisition of rapid running is tentatively interpreted to mean that the drugged Ss evidenced faster acquisition of rapid running in the maze stem than the normal Ss.

Food Intake

Food consumption was significantly reduced by P-CPA treatment. This finding does not necessarily imply that food motivation was reduced by the drug. Miller (1956) has shown that food consumption is only poorly related to food motivation level (deprivation time) and food intake may even be negatively related to deprivation time (Bousfield and Elliot, 1934).

The fact that P-CPA had no effect on asymptotic first trial running times is relevant to the question of drug induced motivational deficits. If P-CPA had reduced hunger motivation, one would expect the drugged Ss to have run slower than nondrugged Ss. Running times have consistently been shown to reflect the food motivation level of rats in mazes (Barry, 1958; Campbell and Kraeling, 1954). The failure to find a P-CPA effect on asymptotic running times implies that the drug did not affect hunger motivation.

It is unlikely that the observed food intake reduction merely reflected a general malaise produced by P-CPA. Only 9 of the 22 P-CPA Ss showed evidence of drug induced malaise (diarrhea, or excessive weight loss) and required antibiotic injections. The other 13 P-CPA treated Ss showed no symptoms of P-CPA treatment. When the 9 P-CPA rats requiring antibiotics were compared with the other P-CPA Ss on food intake during acquisition phase 2, the antibiotic treated rats tended to eat less than the P-CPA rats not treated with antibiotics, but the difference in food intake between the two drug subgroups was not statistically significant. Even the 13 P-CPA Ss that showed no obvious symptoms of P-CPA malaise and did not require antibiotics ate significantly less than the control Ss during acquisition phase 2. Apparently, the reduced food intake following P-CPA treatment was due primarily to P-CPA per se, rather than to drug induced malaise or antibiotic treatments.

The reduction of food intake following P-CPA treatment is probably related to serotonin depletion, but a cautious interpretation of food intake changes is appropriate. There is still some question whether

P-CPA treatment affects brain norepinephrine levels. Even if NE levels are slightly changed by P-CPA treatment, it is much more likely that the food intake changes observed in this study were due to the drastic depletion of serotonin that occurred, rather than to slight depletion or elevation of brain norepinephrine that may have occurred. The fact that the hypothalamus, a major brain site for the control of food intake, is rich in serotonin (Amin, Crawford, and Gaddum, 1954) suggests a possible mechanism for the reduced food intake in this study; that is, hypothalamic functioning may be impaired by serotonin depletion.

Alternation Performance

Previous studies of reward contingent alternation have used absolute alternation performance levels as an indicant of the effectiveness of the reward contingency. This is a questionable tactic. High alternation levels do not necessarily indicate a high effectiveness of the reward contingency (good learning and memory). Spontaneous alternation levels have been reported (Walker, 1956) which are higher than the reward contingent alternation levels obtained by Petrinovich and Bolles (1957).

Similarly, low alternation levels do not necessarily indicate low effectiveness of the reward contingency. Partial rewards (Adkins, Hilles, Weisbrod, and Emmens, 1959), long intertrial intervals (Heathers, 1940) and large rewards (Fowler, Blond, and Dember, 1959) have been shown to reduce spontaneous alternation. All these factors operated in the present study and probably contributed to the low levels of reward contingent alternation observed. The use of the noncontingent

reward yoked group allows the evaluation of the effects of the reward contingency with all other factors held constant. Such a procedure seems the only solution to the problem of evaluating the effectiveness of a reward contingency in an alternation task in light of the many variables that might affect absolute alternation levels.

Phase 1 Alternations. There was no facilitation of alternation behavior by contingent reward during the 30 min. ITI phase of this experiment. Even the absolute alternation levels were low during the 30 days of training with the 30 min. ITI. The poor alternation performance observed with the 30 min. ITI is surprising, considering the high performance levels obtained by Petrinovich and Bolles (1957) with a similarly long ITI. Even the form of the curve observed in phase 1 of this experiment was fundamentally different from that observed by Petrinovich and Bolles (1957). In the present study, Ss showed initially high alternation levels which subsequently declined as acquisition training progressed. Alternation levels then increased slowly to 30 to 35% by the thirtieth day of training. In contrast, Petrinovich and Bolles found initially low alternation levels (less than 10% alternations) which increased to 50 to 60% levels within 9 days of training and then increased further to 60 to 70% alternation levels within 24 days of training.

There are three important procedural differences that might account for the differences in alternation performance observed in this and earlier studies of conditioned alternation. These differences are in the preliminary training methods used, the precautions taken to eliminate

confounding odor cues, and the type of apparatus used. With regard to preliminary training procedures, Petrinovich and Bolles gave rewarded goal placements and straight runway experience preliminary to the alternation training. Only goal placements were given preliminary to training in the present study. Perhaps the straight runway experience given by Petrinovich and Bolles facilitated the development of position habits by precipitating rapid running in the maze, and consequently Ss became less attentive to the alternative choice. Position habits, by being consistently nonrewarded, would also extinguish earlier. By facilitating the rapid development of position habits during early acquisition, preliminary straight runway training might reduce the duration of position habit responding and thereby produce earlier alternation learning.

Another procedural difference that might account for the large performance differences is that while Petrinovich and Bolles did not attempt to eliminate odor cues, in this experiment an attempt was made to attenuate or remove such cues by continuously moving the air in the maze with exhaust fans mounted below the goal boxes. The importance of odor cues in producing unconditioned alternation behavior has recently been demonstrated by Richman, Juels, Peckinpaugh, and Stutz (1969). They observed high levels of spontaneous alternation (79% and 72% in two groups) when S's odors from the previous trial were allowed to persist in the choice apparatus, but low alternation levels (46% and 44%) when odor traces were removed or attenuated between trials. Perhaps part of the high alternation performance observed by Petrinovich

and Bolles (1957) is attributable to frequent spontaneous alternations based on odor cues.

The final procedural difference between the present and earlier studies of conditioned alternation is in the apparatus used. The present study employed a U-maze while in earlier experiments a T-maze was used. Conditioned alternation in the U-maze may be more demanding and difficult than conditioned alternation in the T-maze. In the U-maze, two turns are required to get S from the maze stem to either of the goal boxes (see Figure 1), while in the T-maze only one turn is required. The added complexity should make conditioned alternation in the U-maze very sensitive to changes in neural processes.

Whatever the cause of the performance differences between this and earlier conditioned alternation studies, it is important to note that reward which is contingent upon alternation will not necessarily produce high alternation levels in every situation, even a situation that closely resembles that of Petrinovich and Bolles (1957).

Phase 2 Alternations. When the ITI was reduced from 30 minutes to 30 seconds, all groups, including the yoked group, showed an increase in alternation performance. The increased alternation performance of the yoked Ss when the ITI was reduced must reflect a change in factors governing "spontaneous" alternation rather than factors governing conditioned or reward contingent alternation. However, the contingent reward Ss evidenced the elevated performance throughout phase 2 of the experiment while the alternation levels of the yoked Ss declined as phase 2 training progressed. This finding suggests that

there was an improvement in reward contingent alternation performance which was permanent, as well as a transient increase in unconditioned alternation as a result of reducing the ITI.

There was no effect of P-CPA on reward contingent alternation performance during acquisition phase 2 of this experiment. The fact that P-CPA did not disrupt phase 2 alternation has important implications. The result indicates that the serotonin depletion that resulted from P-CPA treatment did not disrupt memory processes, insofar as memory processes contributed to conditioned alternation performance, and that the serotonergic fibers that course throughout the limbic system and neocortex do not mediate the memory functions that have been ascribed to these structures.

It might have been expected, on the basis of Essman's suggestion that the amnesic effects of electroconvulsive shock are due to elevated 5HT levels, or on the basis of the previously observed facilitation of performance in learning tasks that sometimes results from P-CPA treatment, that the 5HT depleted Ss would perform at higher alternation levels than normals. No facilitation of alternation performance following P-CPA was observed. Apparently, serotonin depletion does not improve memory processes.

The phase 2 alternation results were essentially the same for those P-CPA treated Ss that showed symptoms of P-CPA malaise and those P-CPA treated Ss that did not show signs of malaise. Reporting separate data analyses for the P-CPA treated Ss that showed symptoms of malaise and for the P-CPA Ss that did not show symptoms of malaise might be

very helpful in interpreting experimental observations of P-CPA produced performance deficits by other investigators. Previous experimenters have apparently not been concerned with the behavioral consequences of P-CPA-induced malaise, above and beyond the effects of the biochemical changes the drug produces. But general malaise could be responsible for the behavior deficits that have sometimes been observed following P-CPA treatment, and the deficits may have nothing to do with the effects of P-CPA on serotonin or phenylalanine.

An Hypothesis of the Role of Brain Serotonin

The failure to find a P-CPA deficit in alternation performance indicates that serotonin does not have a role in information storage. But the results of several recent experiments suggest that endogenous serotonin may have a fundamental role in sensory processing. Tenen (1967) and Lints and Harvey (1969) found decreases in shock thresholds after P-CPA treatment of rats. Brody (1968) observed that P-CPA treated rats were more reactive to visual and auditory stimuli than normal Ss. Taste thresholds have apparently been lowered with P-CPA (Nance and Kilbey, 1970; Brody, 1969). P-CPA has been shown to increase audiogenic seizure susceptibility of mice (Schlesinger, Schreiber, Griek, and Henry, 1969). These results all suggest that serotonin may normally function to reduce sensory input. When serotonin is depleted, sensitivity to arousing stimuli is increased. The speculation that serotonin normally inhibits sensory input would explain the insomnia and excessive responsiveness to sexually arousing stimuli that results from serotonin depletion with P-CPA.

A possible mechanism for the hypothesized role of serotonin in the reduction of sensory input has been suggested by Eidelberg, Goldstein, and Deza (1967). Eidelberg, et. al., found that i.v. injections of 5HTP, the precursor of serotonin, lowered single unit firing rates, and they hypothesized that serotonin is an inhibitory transmitter substance. This hypothesis is a slightly more refined form of the Brody and Shore (1956) speculation that serotonin is a chemical transmitter which mediates parasympathetic brain activity. Weight and Salmoiraghi (1968) and Krnjevic and Phillis (1963a) reviewed several studies which indicate that the most frequently observed effect of 5HT or its precursor on limbic and cortical neurons is inhibition. The observation of Anden, et. al., (1964) that i.v. injections of 5HTP reduced afferent potentials in the cat spinal cord is perfectly consistent with the hypothesis that 5HT normally functions to inhibit sensory neurons.

Some conjecture about the full significance of the serotonergic inhibition of CNS neurons is appropriate. Both serotonergic (Dahlström and Fuxe, 1964) and cholinergic fibers (Snell, 1961) course through the midbrain tegmentum (Reticular Activating System, RAS). Stimulation of the midbrain produces a release of forebrain 5HT (Aghajanian, Rosecrans, and Sheard, 1967) and of acetylcholine (Kanai and Szerb, 1965). Acetylcholine activates central nervous system neurons (Krnjevic and Phillis, 1963b), while 5HT inhibits central neurons (Krnjevic and Phillis, 1963a).

The above facts are directly relevant to the hypothesized role of 5HT in the inhibition of sensory units. The reticular formation is thought to control sensory inputs and the responsiveness (arousal) to external cues. It seems quite possible that the modulation of sensory inputs by the RAS is mediated by both the cholinergic and serotonergic fibers that lie within the midbrain tegmentum. The cholinergic fibers may facilitate the transmission in sensory neurons while the serotonergic neurons may inhibit sensory neurons. Serotonergic neurons may be indispensable in the normal reduction of sensory sensitivity, as in adaptation.

If the cholinergic and serotonergic fibers in the brainstem do compose two antagonistic systems controlling responsiveness to external cues, some interesting predictions about drug effects can be made. P-CPA, because it depletes serotonin, should lower sensory thresholds in the various sensory modalities. It would further be expected that substances which elevate serotonin levels would raise sensory thresholds. Similarly, cholinergic stimulation should lower thresholds while cholinergic blockade could be expected to raise thresholds. A more complex prediction arising from the hypothesis that antagonistic serotonergic and cholinergic systems control sensory inputs is that the effects of anticholinergic drugs like scopolamine and atropine on sensory phenomena should be potentiated by serotonergic elevation with 5HTP.

Also, performance in various tasks requiring "attention" to low intensity external cues should be facilitated by cholinergic excitation and/or by serotonin depletion, but performance on the same tasks should

be disrupted by cholinergic blockade and/or 5HTP injections which elevate serotonin levels.

While there has been little experimental work analyzing sensory threshold changes following cholinergic and serotonergic manipulation, the findings of Cicero (1969) are relevant to the question of sensitivity changes following cholinergic stimulation and serotonin depletion. Cicero found that rats reduced their ethanol intake in response to chemical stimulation of the brain with carbachol and following serotonin depletion with P-CPA. The decrease in ethanol consumption following cholinergic stimulation (carbachol) and serotonin depletion (P-CPA) may be caused by increased gustatory sensitivity to ethanol. If so, Cicero's findings are perfectly consistent with the hypothesized cholinergic-serotonergic control of sensory input.

Further research analyzing the effects of cholinergic and serotonergic manipulation on sensory thresholds may be expected to glean considerable information concerning the functional roles of the hypothesized neural transmitter substances, serotonin and acetylcholine.

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