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The Effects of Para-Chlorophenylalanine on a Conditioned Emotional Response in Rats

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This Thesis for the Master of Science Degree

by

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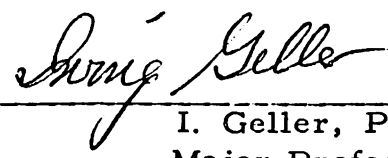

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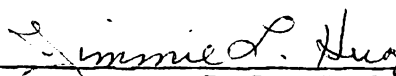

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INTRODUCTION

The central nervous system contains a number of chemical substances which have been implicated in many mental and neurological processes. Those substances which have been studied extensively and are considered to be most significant include acetylcholine, norepinephrine, epinephrine and serotonin.

Acetylcholine, first synthesized by Baeyer in 1867, was for many years of little interest. In 1921, Otto Loewi established the first real proof of the chemical mediation of nerve impulses by the peripheral release of a specific chemical agent. Five years later, Loewi and Navratil presented evidence for the identification of this agent as acetylcholine (1). Acetylcholine also has been positively identified as a transmitter substance in the central nervous system (CNS) (2).

In some areas of the CNS where minimal amounts of acetylcholine are present, other transmitter substances have been identified. They are serotonin and norepinephrine, two biologically active amines unevenly distributed throughout the brain.

Serotonin, 5-hydroxytryptamine (5-HT) has been known, especially to mammalian physiologists for almost a century, as a potent

vasoconstrictor substance present in blood platelets. Until the late 1940's serotonin was known by a variety of names, of which "vasotonin" was the most common. In late 1949, the introduction of synthetic serotonin led to a great deal of research with this compound (3). There have been many speculations with regard to serotonin's possible physiological functions; one of the most challenging characterizes serotonin as a neural transmitter substance.

Studies dealing with the metabolism of serotonin in the brain have demonstrated that it is synthesized from the amino acid 5-hydroxytryptophane, (5-HTP). An enzyme, monoamine oxidase, acts on serotonin as a substrate and seems to be responsible for its degradation. It is then excreted in the urine as 5-hydroxyindoleacetic acid, (5-HIAA) (4). Administration of large doses of serotonin's precursor, 5-HTP, to animals, increases brain serotonin concentrations and causes generalized somatic, autonomic and behavioral reactions (5).

The greatest concentrations of serotonin are found in the more primitive areas of the brain, primarily the midbrain and the hypothalmus. Presence of this substance can be demonstrated by spectrophotofluorimetry and by bioassay procedures (6).

Although it has been postulated that the amount of serotonin in the brain may be related to the symptomatology of mental illness, its role is not too clear in this regard. Data derived from animal experiments have shown that antimetabolites of serotonin such as

LSD and Yohimbine caused "mental aberrations" (7). Some investigators believe that schizophrenia is caused by a serotonin deficiency while others maintain that it is an excess of serotonin that causes the characteristic symptoms (8).

One approach toward clarification of the possible role played by the neurotransmitter substances in normal or abnormal behavior, is through experimental manipulation of these substances in behavioral studies with animals.

During the past forty years, psychologists have been perfecting techniques for studying various kinds of behavior in laboratory animals. They have shown that the characteristics of an organism's behavior are, to a considerable extent, determined by what the environmental consequences of that behavior have been in the past. The process of manipulating behavior as a function of the behavior's environmental consequences has been termed "operant conditioning" (9). Psychologists have successfully applied operant conditioning techniques to the study of many different types of behavior in animals. First, the response selected for measurement and manipulation is one that is reproducible between species and that the experimental organism can make rapidly, easily and repeatedly. A second requirement is the selection of an environmental consequence, or "reinforcement," appropriate to the particular experimental animal, and the use of motivational levels that are strong enough to minimize the effects of any irrelevant variables.

Finally, an additional aspect of this approach is the systematic limitation of the experimental environment, to permit at least some reasonable degree of stimulus control and specification. For example, the subject in this type of study may be a hungry or thirsty rat pressing a bar in a small chamber to obtain a small bit of food or a drop of water. Programming of the experimental procedures and recording of the animal's behavior is accomplished automatically by timers, magnetic counters, cumulative work recorders, and associated relay circuits. In this manner, one can place some arbitrary sample of behavior under experimental control so that behavioral processes may be investigated as a function of a wide variety of operations including neurophysiological manipulations, "emotional" disturbance, and even drugs.

Utilizing operant conditioning techniques, investigators have conditioned in animals, behavior motivated by "fear" or "anxiety".

> "Anxiety has at least two defining characteristics (1) it is an emotional state, somewhat resembling fear, and (2) the disturbing stimulus which is principally responsible does not precede or accompany the state but is "anticipated" in the future."

The "anxiety" response which has been referred to as a Conditioned Emotional Response (CER), is established in laboratory animals as follows: (11) The food deprived subject is placed in a skinner box and trained to press a lever in order to obtain a liquid or solid food reward. After the subject has learned to press the lever on an intermittent

reward schedule, an auditory or visual stimulus, (CS), is introduced for a given period of time during the lever-pressing session. The stimulus (light or tone) is terminated with a shock, (US), of moderate intensity and short duration, delivered to the animal's feet through the grid floor of the experimental chamber. After a series of CS - US pairings, the CER appears as a suppression of the animal's ongoing lever-pressing behavior. This is accompanied by autonomic disturbance manifested by defecation, piloerection and the subject freezing motionless in a corner of the chamber during subsequent presentations of the CS.

There are now available a number of chemical agents which act selectively on brain substances. Of particular interest are those that inhibit the synthesis of monoamines. One of these, p-chlorophenylalanine, (p-CPA), selectively inhibits the synthesis of serotonin. In 1964, B. Kenneth Koe of the Pfizer Medical Research Laboratories, discovered the peculiar inhibitory properties of p-CPA (12). This investigator first attempted to determine if p-CPA released serotonin from its storage sites or if it blocked its biosynthesis (13). If p-CPA released serotonin, one would expect to find in tissues, large quantities of its major metabolite, 5-hydroxyindoleacetic acid, (5-HIAA). However, it was found that p-CPA greatly reduced 5-HIAA levels. Koe also pointed out that p-CPA only slightly depleted stores of the catecholamines, norepinephrine and dopamine. Reserpine, a drug known to decrease serotonin content in tissues by releasing it from storage

sites, releases catecholamines as well. Koe's evidence strongly suggests that p-CPA blocks the biosynthesis of serotonin since p-CPA blocked increases in levels of serotonin and 5-HIAA which normally occur following administration of tryptophane, the serotonin precursor. p-CPA also blocked the increase in serotonin levels which occur when monamine oxidase inhibitors are given to animals. Finally, when p-CPA was given to rats or monkeys, their livers and brains were unable to convert tryptophane, the immediate precursor of serotonin, to 5-HIAA. Tryptophane Hydroxylase, the enzyme required for this conversion, was inhibited after p-CPA and the time course of the enzyme blockade in the animal closely paralleled the reduction in brain serotonin. Although no one has as yet isolated an active metabolite of p-CPA in the body, evidence suggests that a much more active substance is formed and acts on serotonin. p-CPA is far more effective in living animals than it is with in vitro enzyme preparations. This suggests that something is formed in the body that is not found in in vitro preparations. Secondly, concentrations of serotonin reach their lowest points long after p-CPA has dropped from its highest concentrations in the body.

Some work has been published about the effects of p-CPA on behavior. Stevens, Resnick and Krus (1967) reported that p-CPA administration to rats and concomitant reduction in brain serotonin, resulted in facilitation of learning in maze discrimination tasks (14). Brody (1970) reported that administration of p-CPA made rats more

"reactive" to external stimulation (15). He also reported that p-CPA increased the number of punishments thirsty rats took in order to obtain water. Robichaud and Sledge (1969) demonstrated that p-CPA produced an extensive attenuation of a conditioned suppression of operant behavior maintained by punishment (conflict behavior) (16). Geller and Blum (1970) confirmed the findings of Robichaud and Sledge (17). They reported that p-CPA reinstated lever-pressing behavior that had been suppressed by punishment. Repletion of serotonin by administration of 5-HTP, the serotonin precursor, resulted in the return of the conditioned suppression to pre-p-CPA control levels.

The conditioned suppression technique used by these investigators has been referred to as "conflict behavior." The suppression of "conflict" was superimposed on the hungry animal's normal lever-pressing behavior as follows; during the experimental session, a tone of three minutes duration was introduced as a signal that all lever presses would be rewarded with food in contrast to no-tone periods where lever responses were rewarded only intermittently. However, a punishment contingency was also in effect during the tone periods so that every lever press not only produced food reward, but also a shock to the animal's feet through the grid floor of the skinner box. This resulted in a suppression of lever-pressing behavior during tone periods. The conflict suppression differs from conventional CER in that rats have the option of accepting shocks during the tone periods while CER animals cannot avoid the single shock which terminates each tone period.

Also, the symptoms manifested during CER conditioning would suggest an underlying "anxiety" characterized by autonomic disturbance.

The initial intent of this study was to investigate p-CPA's actions on conditioned "anxiety" (CER) in rats in order to ascertain whether the derived effects were qualitatively similar to those previously reported for "conflict" behavior. Specifically, the goals of this study were to determine if p-CPA would reinstate lever-pressing behavior that had been suppressed by a series of tone-shock pairings. If a change in behavior did occur, would the reintroduction of serotonin, through the use of 5-HTP, return the behavior to pre-drug control levels.

MATERIALS AND METHODS

Subjects

Ten male rats of the Holtzman Sprague Dawley strain approximately 90 days old were used as subjects. All subjects were reduced to 80% of their original starting body weight and maintained at this weight throughout the experiment by limited feedings after each experimental session.

Apparatus

The apparatus consisted of a conventional skinner box, a sound resistant cubicle which contained a lever on the front wall, an automatic feeding device for the delivery of a liquid food reward, a small speaker for the presentation of auditory stimuli and a grid floor for the delivery of electric shocks. Programming and recording of the experiment was accomplished automatically by timers, magnetic counters, a cumulative work recorder and associated relay circuits. White background noise was piped into the experimental chamber in order to mask any extraneous stimuli. A photograph of a major portion of this equipment is shown in Figure 1.

Procedure

Experimental sessions of 30 minutes duration were conducted on

Monday through Friday of each week. The rats, 23 1/2 hours food deprived, first were trained to press the lever for a liquid food reward which was obtainable on a two-minute variable-interval schedule of reinforcement (on the average, once every two-minutes) (18). When the lever-pressing rates became relatively stable, a clearly audible but non-aversive 1800 cycle/sec. tone of three-minutes duration was introduced during the first fifteen minutes of the session. The tone was terminated with a 40 volt shock of .25 seconds duration delivered to the animal's feet through the grid floor of the apparatus.

A record was kept of the number of lever responses made by the rats during the three-minute tone periods and the three-minute periods prior to the tone onset. The degree of conditioned suppression was calculated as a ratio of response output during the three-minute tone period to the response output during the three-minute pretone period. This is referred to as the subject's suppression ratio, and is calculated according to the following formula:

$$\text{suppression ratio} = \frac{\text{tone responses}}{\text{pre-tone responses}}$$

No suppression of lever pressing during the tone periods was reflected by suppression ratio values of 0.75 or more, moderate suppression by values approximating 0.50, and high suppression by values of 0.10 or less. When the subjects attained a suppression ratio of .10 or less, the drug phase of the experiment was begun.

Preparation and Administration of Drugs

Rats were given 0.5% Carboxymethylcellulose (CMC), p-CPA, 5-HTP or p-CPA plus 5-HTP in a mixed order so that the sequence of administrations differed between subjects. All doses were calculated as mg. base/kg. A minimum of one month elapsed between p-CPA or 5-HTP administrations, in order to assure that the effects of either agent used previously had dissipated completely.

The dl form of p-CPA was prepared as a suspension in CMC and administered orally at 320 or 400 mg/kg. on the evening prior to an experimental session, to rats whose suppression ratios were 0.10 or less. The 400 mg/kg dose of p-CPA was used only for rat P-3, since the 320 mg/kg. dose was ineffective. CMC was administered orally in a volume equal to that of the p-CPA dose for each subject also on an evening prior to an experimental session. The dl form of 5-HTP was prepared as a saline solution and administered intraperitoneally at 15 to 40 mg/kg., two to six hours prior to the experimental session. Since the most effective dose and time of administration for 5-HTP was not known, they were different for each subject. However, when 5-HTP was given alone, the dose and time course used for each rat was the same as when the 5-HTP followed the p-CPA treatment for that animal.

RESULTS

In Table 1 are shown suppression ratio data for rats after CMC or 5-HTP. The suppression ratio values which range from 0.00 to 0.09 indicate virtually complete suppression of responding during tone periods under control or 5-HTP, CMC conditions. Both of these agents when administered alone had no effect on the CER.

Figure 2 illustrates what is typically seen when p-CPA is given alone. Each cumulative response record shows the pre-tone, tone, and post-tone responses for Rat P-11. The pen offsets indicate the three-minute tone period and the number above the line indicates the suppression ratio for the trial. On the control run, P-11 made no responses during the tone period. Twenty hours, or one day after the p-CPA was given, lever pressing was still suppressed during the tone period. Two days or 44 hours post drug shows a suppression ratio of .25 which indicated that the rat's responses during the tone period was one-fourth the amount made during the pre-tone period. At 68 hours or three days post-drug, maximum responding occurred during the tone. The suppression ratio of .62 indicates that during the tone period the rat made 62% of the total responses made during the pre-tone period. By 92 hours or four days post-drug the suppression ratio of zero indicates complete

suppression during the tone just as on the pre-drug control level.

Figure 3 illustrates the effect of p-CPA and 5-HTP on the CER. Each cumulative response record shows the pre-tone, tone and post-tone responses of the subject, and the numbers above the line are suppression ratios. Rat P-4, had a control run suppression ratio of 0. Twenty hours after p-CPA the suppression ratio for rat P-4 increased from a control value of 0 to .40. Immediately after this trial the subject was taken out of the box and injected with 5-HTP at 15 mg/kg. Two hours after 5-HTP the suppression was reinstated, as shown by the decrease in suppression ratio to .17. As the effects of the 5-HTP, dissipated, 26 hours post administration, the p-CPA effects were again seen and suppression was attenuated. At 68 hours the suppression ratio had reached a peak of .85. After ninety two hours the p-CPA effects had dissipated completely and the suppression ratio was back near its pre-drug control level of zero.

Data for Rat P-9 shown in figure 4, illustrate both the effects of 5-HTP alone and 5-HTP plus p-CPA on the CER. 5-HTP alone at 20 mg/kg. was without effect on the CER and when the 5-HTP followed the p-CPA trial there was no change in suppression ratio. Under 5-HTP the absolute response rates of the tone and pre-tone periods decreased, although the relative response rates remained approximately the same. Possibly the 20 mg/kg. dose of 5-HTP was too large for this animal and resulted in a general depression of all responding thereby preventing

accurate measurement of reversal of p-CPA's attenuating effect.

Table 2 shows the effect of p-CPA on the CER for four rats. For all subjects p-CPA increased suppression ratios, indicating an attenuation of the CER. The highest suppression ratio for these subjects, occurred between 20 and 68 hours post drug, with the drug effect lasting between 68 and 92 hours.

In Table 3 are shown the effects of p-CPA and 5-HTP on the CER in rats. The numbers, unless otherwise indicated, represent suppression ratios for each trial. The underlined numbers show suppression ratio values for those trials which followed the administration of 5-HTP. Within 68 hours post p-CPA, suppression ratios for each rat were higher than during control. Following 5-HTP administration, suppression ratios were lowered in varying degrees for eight out of ten animals during the trial following 5-HTP administration. In two rats there was no change in the suppression ratio from that before the 5-HTP administration.

DISCUSSION

Administration of 5-HTP alone was generally without effect on conditioned suppression. Green and Sawyer (1964) reported that doses of 5-HTP smaller than 25 mg/kg. injected intraperitoneally, did not produce a significant increase in brain 5-HT concentration (20). This lack of change in brain concentration might account for the lack of effect on conditioned suppression. For Rat P-6 the slight increase in the suppression ratio following the 40 mg/kg. dose of 5-HTP might be due to an increase in brain 5-HT resulting from this large dose of 5-HTP; it also could have been due to a day to day minor variation in the subject's response rate.

Findings of this experiment, showing that a suppression of lever-pressing behavior of the CER type, may be reinstated by administering p-CPA, are qualitatively similar to those previously reported by Geller and Blum for a conditioned suppression based on punishment. Since 5-HTP reversed the p-CPA effects, they stated that the p-CPA effects on behavior were probably due to a selective depletion of brain 5-HT.

In the present study, reversal of p-CPA effects with 5-HTP was not obtained in two out of ten rats, while for several other animals the suppression was reinstated permanently after 5-HTP. Therefore, it is

not known whether the reinstatement of the suppression following 5-HTP was due to serotonin repletion or simply a return of the suppression to pre-drug control values following the dissipation of p-CPA effects.

The absence of a clear-cut reversal of p-CPA effects on the CER with 5-HTP, are not in agreement with those previously reported for conflict behavior by Geller and Blum. In every case they obtained an attenuation of conflict behavior in p-CPA pre-treated animals following 5-HTP treatment.

This does not imply that p-CPA's behavioral effects are not due to its selective depletion of brain 5-HT. There are basic differences between the two experiments that might account for these differences and some background information to support it. The relationships between brain serotonin, norepinephrine and behavior were the subjects of a recent investigation by Miller and Maickel (1969) (21). Their findings suggest that the balance between free serotonin and norepinephrine is related to behavioral disturbance. They demonstrated that when free 5-HT was greater than free NE, rats did not avoid an electric shock as well as they did when free 5-HT was less than free NE. It has also been reported by Bliss and Zwanziger that shocks to the feet of guinea pigs and rats are associated with drops of 10 to 30% of brain norepinephrine while serotonin remains stable.

Since after receiving p-CPA, the rats in the conflict experiment take many more shocks than rats in the CER procedure, norepinephrine

levels are probably reduced to a much greater degree in "conflict" than in CER. The absence of the reversal with 5-HTP in the present CER experiment, might be due to the fact that a greater amount of 5-HT replacement was needed, to restore the 5-HT - norepinephrine balance. Data for Rat P-6 lend support to this speculation. Following p-CPA, 25 mg/kg. of 5-HTP produced no drop in suppression ratio; however, increasing the 5-HTP dose to 40 mg/kg., did result in a complete and total drop in suppression ratio.

Table 1

Effects of 5-HTP or CMC on a CER in Rats

Rat	Dose mg/kg.	Time Prior to Testing	Suppression Ratios			
			Control	5-HTP	Control	CMC
P-1	25	2 hours	0.00	0.00	0.07	0.04
P-3	25	2 hours	0.05	0.07	0.05	0.07
		4 hours	0.05	0.02		
P-4	15	2 hours	0.00	0.04	0.04	0.02
P-6	25	2 hours	0.03	0.01	0.00	0.02
	40	2 hours	0.01	0.09		
P-7	15	2 hours	0.01	0.00	0.06	0.07
	20	2 hours	0.07	0.08		
P-8	25	2 hours	0.01	0.01	0.03	0.05
P-9	20	2 hours	0.00	0.01	0.04	0.04
P-10	25	6 hours	0.01	0.01	0.00	0.00
P-11	25	2 hours	0.05	0.02	0.01	0.02
P-12	25	6 hours	0.02	0.05	0.04	0.04

Table 2

Effects of p-CPA on a Conditioned Emotional Response in Rats

Rat # -	1	3	7	11
Suppression Ratio Before Drug	0.01	0.00	0.01	0.00
Hours to Maximum Drug Effect	68	20	68	68
Suppression Ratio at Maximum	.38	.54	.57	.62
Hours Until Drug Effects Dissipated	92	68	92	92
Suppression Ratio-Post-Drug Control	.08	.03	.06	.00

Table 3

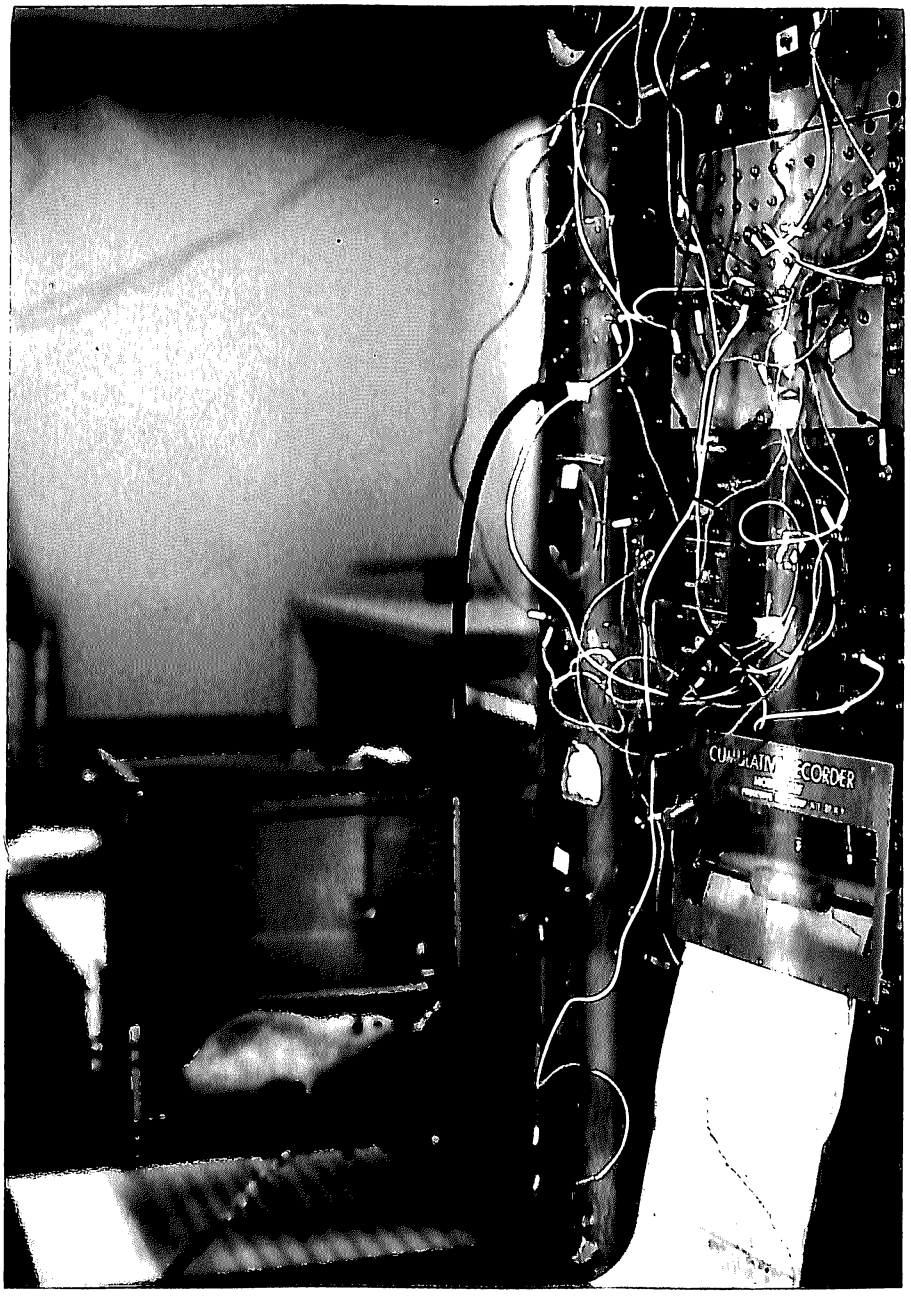
Effects of 5-HTP on p-CPA Attenuation of CER

Rat	p-CPA mg/kg.	5-HTP mg/kg.	Hours Post p-CPA Control	20	44	68	92	116	140
P-1	320	25	0.01	0.00	0.20, 0.00	0.01	0.01	0.00	0.00
P-3	400	25	0.00	0.26	0.56, 0.66 0.05*	0.04	0.04	0.06	0.02
P-4	320	15	0.00	0.40, 0.17	0.26	0.84	0.05	0.04	0.01
P-6	320	25/40x	0.08	0.04	0.77, 0.81	1.00, 0.00	0.01	0.07	0.00
P-7	320	15/20x	0.02	0.58	0.85	1.00, 1.00	0.22	0.79, 0.96	0.62
P-8	320	25	0.04	0.15	0.06	0.86, 0.12	0.64	0.01	0.00
P-9	320	20	0.00	0.13	0.67	1.00, 1.00	0.05	0.08	0.05
P-10	320	25	0.04	0.30	0.06	0.37	1.00, 0.04	0.00	0.01
P-11	320	25	0.01	0.04	1.00, 0.81	0.03	0.04	0.07	0.02
P-12	320	25	0.03	0.14	0.14	1.00, 0.24	0.13	0.06	0.03

*Rat P-3, represents a second trial given 4 hours after 5-HTP administration

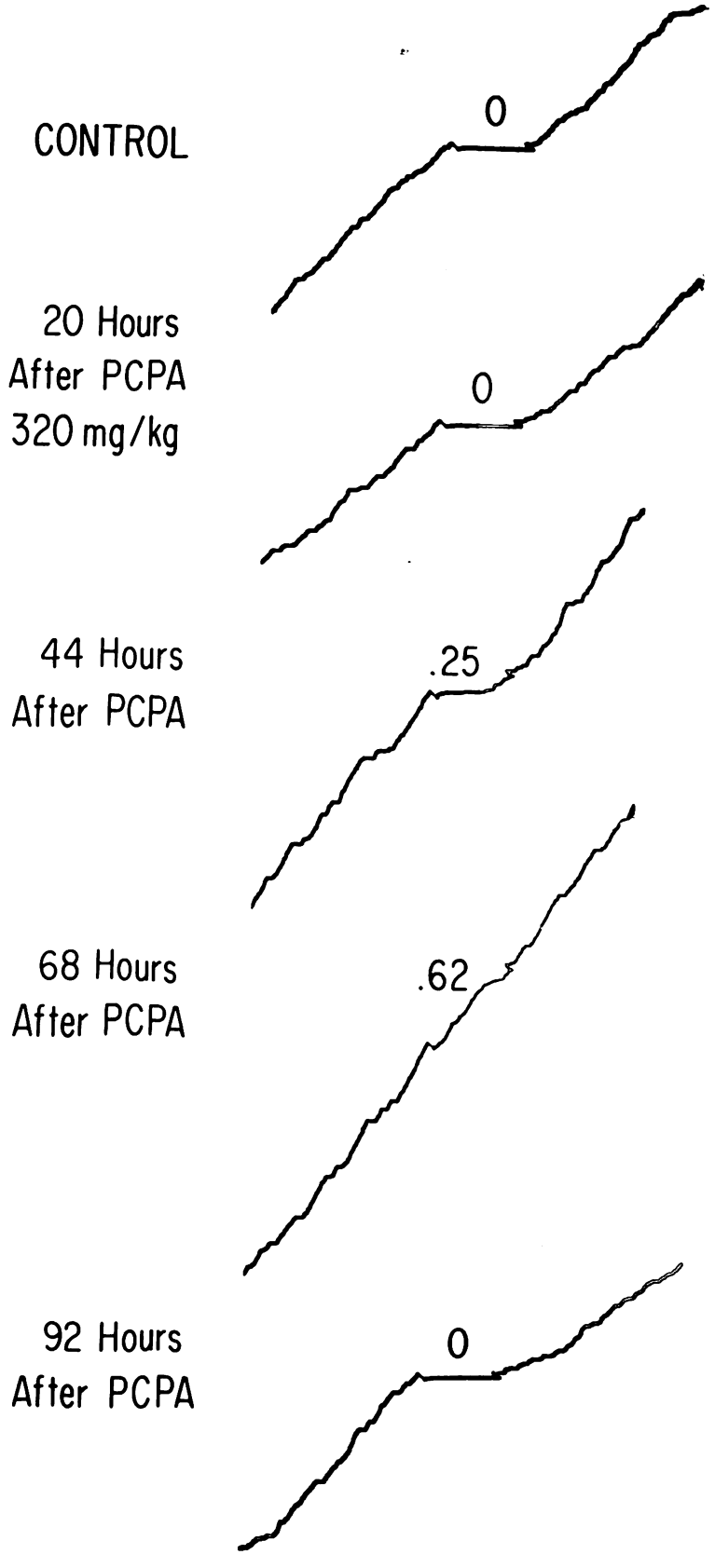
xThese rats were administered a second dose of 5-HTP since the first dose appeared to be ineffective

Figure 1



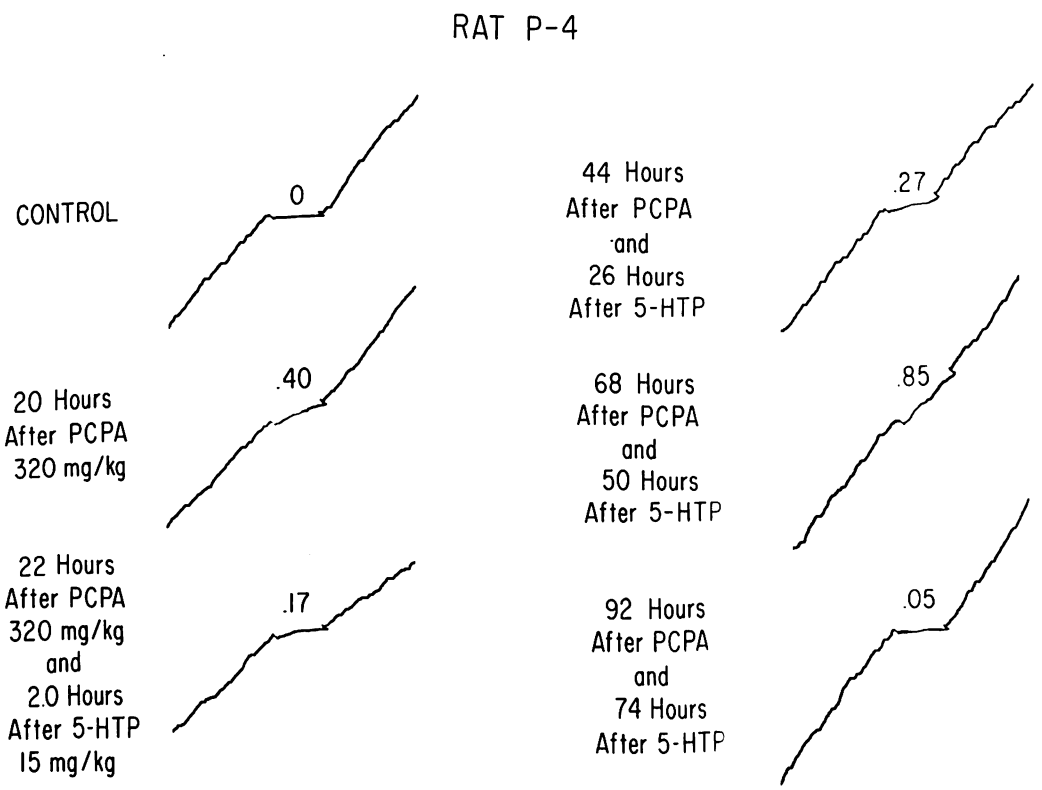
Skinner box and control equipment used in the experiment

Figure 2



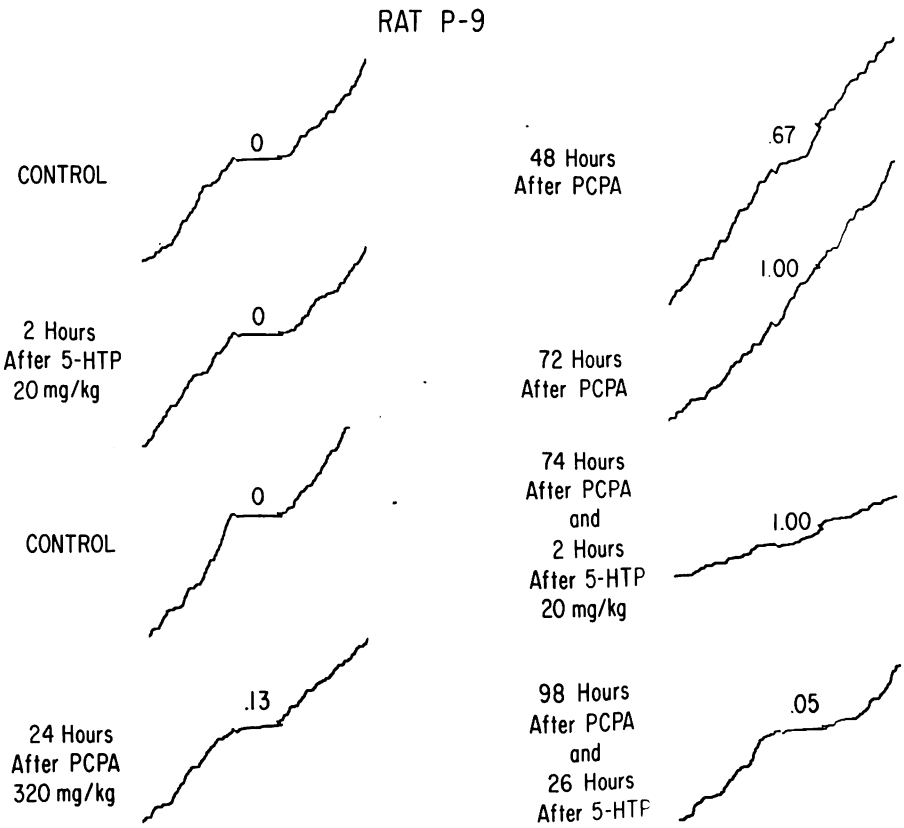
Cumulative response records for Rat P-11, showing the effects of p-CPA on the CER. The pen offsets indicate the tone periods. Suppression ratios are shown by the numbers above the records

Figure 3



Cumulative response records showing the effects of p-CPA and 5-HTP on a CER. The pen offsets indicate the tone periods. Suppression ratios are shown by the numbers above the records.

Figure 4



Cumulative response records showing the effects of 5-HTP alone and p-CPA plus 5-HTP on a CER. The pen offsets indicate the tone periods. Suppression ratios are shown by the numbers above the records.

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