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Learning Associated with the Amphetamine-State: Role of Brain Amines

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LEARNING ASSOCIATED WITH THE AMPHETAMINE-STATE: ROLE OF BRAIN AMINES

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DEDICATION

The author would like to dedicate this thesis to his parents, Mr. and Mrs. Saul Tobias, without whose encouragement, confidence and prayers this graduate training might not have been possible.

ABSTRACT

Behavior is under the control of external and internal stimuli. The emission of behavior is therefore most efficient when the stimulus-conditions similar to those under which the behavior was acquired are reinstated. Amphetamine was shown to produce a "stimulus-state" which controlled behavior. A decrement in response strength occurred when a response acquired under the influence of amphetamine was emitted in the absence of amphetamine or in the presence of no-drug. The response strength recovered when the amphetamine-state was reinstated.

Rats were trained to jump to a wooden platform from an electrifiable grid-floor, in order to avoid shock. A buzzer was used as the conditioned stimulus. Seven days were allowed to elapse between testing and training. A conditioned avoidance response acquired under the influence of amphetamine was emitted without decrement under 1) 2.0 mg amphetamine per kg body weight, 2) 100 or 400 mg dl-3,4-dihydroxyphenylalanine per kg body weight, 3) 50 mg dl-α-methyl-p-tyrosine per kg body weight, 100 or 400 mg dl-3,4-dihydroxyphenylalanine per kg body weight and 2.0 mg amphetamine per kg body weight, 4)

316 mg parachlorophenylalanine per kg body weight, 75 mg dl-5-hydroxytryptophan per kg body weight and 2.0 mg amphetamine per kg body weight.

However, the per cent avoidance deteriorated markedly (all comparisons were statistically significant), when subjects trained under amphetamine were tested after pretreatment under 1) no injections, 2) dl-α-methyl-p-tyrosine and amphetamine, 3) parachlorophenylalanine and amphetamine, 4) hydroxyamphetamine (10 mg/kg or 30 mg/kg), 5) chlorpromazine (1 or 4 mg/kg) and amphetamine, 6) cyproheptadine (10 mg/kg) and amphetamine. Moreover, animals treated chronically with amphetamine, when trained under amphetamine, showed a decrement in response-strength when tested under no-drug.

A conditioned-avoidance response acquired under the influence of hydroxyamphetamine (30 mg/kg) was emitted without decrement in response strength under either hydroxyamphetamine or no-drug. Further, a conditioned avoidance response acquired under the influence of α-methyl-p-tyrosine and amphetamine was emitted without decrement in response strength under either α-methyl-p-tyrosine and amphetamine or just amphetamine.

These studies suggest that there exists a stimulus-state associated with amphetamine and that this behavior-controlling

state is not the result of the stimulant property of the drug.

The drug-interaction studies suggest that central catecholamines and 5-hydroxytryptamine are involved in the amphetamine-state. Further, by varying the concentration of either amine, the amphetamine-state may be altered.

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I. INTRODUCTION

Recent experiments have demonstrated that drugs, acting on the brain, may also serve as discriminative stimuli. A rat may learn to make a certain choice in a maze if it is injected with one drug and a second choice if it is tested with no drug (Overton, 1964, 1966). The learning associated with a drug-state may have important implications to humans. New behaviors, acquired via behavior modification, under the influence of a drug may not be reproduced without benefit of the drug.

Amphetamine, which has recently been shown to produce state-dependent learning (Lal, 1969), has been shown to release central norepinephrine (Glowinski et al., 1966b; Carr and Moore, 1969) and 5-hydroxytryptamine (Beauvallet et al., 1969). Moreover, 5-hydroxytryptophan, a precursor of 5-hydroxytryptamine, has also been shown to cause a release of norepinephrine in vivo (Brodie et al., 1966) and in vitro (Carlsson et al., 1963). In addition, recent evidence indicates that 5-hydroxytryptamine acting through norepinephrine may be responsible for raising the threshold necessary for shock induced fighting (Lal et al., 1969).

The present investigation sought evidence to establish:

1) A behavioral controlling amphetamine-state in rats using a learning criterion which is weaker than that used previously in mice.
2) That the learning associated with the amphetamine-state is not the result of either the stimulant property of the drug or the novelty of the drug stimulus.
3) That the amphetamine-state is due to a central action of amphetamine.
4) The role or roles of norepinephrine and/or 5-hydroxytryptamine in the amphetamine-state.
5) Whether hydroxyamphetamine, a drug which has only peripheral actions (Innes and Nickerson, 1965) produces state-dependent learning.

II. LITERATURE SURVEY

Learning Associated with a Drug-State.

Learning associated with a drug-state has been demonstrated in a number of procedures.

Utilizing pole-climbing avoidance, Otis (1964) demonstrated that chlorpromazine-trained animals showed a decrement in avoidance when tested with saline but not with chlorpromazine. Similarly, saline-trained animals showed a decrement in avoidance when tested with chlorpromazine but not with saline.

Utilizing pit avoidance, Lal (1969) demonstrated that amphetamine-trained animals showed a decrement in avoidance if tested with either chlorpromazine or saline. Similarly, chlorpromazine trained animals showed a decrement in avoidance when tested with either amphetamine or saline.

Overton (1964), utilizing a t-maze, demonstrated that phenobarbital-trained animals showed a decrement in responding when tested under no-drug. He also demonstrated response control in a t-maze using pentobarbital and no-drug (1964), pentobarbital and saline (1964), phenobarbital and saline (1966), atropine and saline (1966), and chlorpromazine and phenobarbital (1966). However, he could not obtain response control under

either gallamine or tetraethylammonium and saline (1964). These latter results indicate that the response control by the barbiturates was not due to either muscle flaccidity or an autonomic blockade.

Utilizing a two-operant schedule, one under the control of positive reinforcement of food and the other under the control of shock avoidance, Kubena and Barry (1969) demonstrated that rats would elicit the same response for high doses of alcohol, pentobarbital, chlordiazepoxide and chloral hydrate in each procedure.

Utilizing a schedule of positive reinforcement, Belleville (1964) demonstrated that rats trained to bar-press for food under saline, amphetamine, or morphine made a greater number of responses during extinction, if they received the same drug that was administered during acquisition. Roffman and Lal (1969)demonstrated that rats, trained to bar-press for water, would make a greater number of responses during the second session of extinction if they had amphetamine on the first extinction session.

Learning associated with a drug-state can thus be demonstrated by the use of either positive or negative reinforcement. Further, stimulants and depressants, neuronal blockers and narcotic analgesics are some of the classes of drugs that produce state-dependent learning.

Relationship Between Amphetamine and Norepinephrine

Amphetamine, which has recently been demonstrated to cause a decrease in the level of brain norepinephrine in fighting mice (Welch and Welch, 1967), has been shown to cause a striking elevation in the level of plasma catecholamines (Harvey et al., 1968). Moreover, after the injection of H^3-norepinephrine into the lateral cerebral ventricle of cats, Carr and Moore (1969) noted that the addition of d-amphetamine to the ventricle perfusion fluid caused a significant increase in the concentration of H^3-norepinephrine and normetanephrine in the effluent of the perfusion fluid. These studies indicate that norepinephrine may be involved in the action of amphetamine.

Utilizing various behavioral tests, Weissman et al. (1966) noted that α-methyl-p-tyrosine, an inhibitor of norepinephrine synthesis (Spector et al., 1965) antagonizes the hyperactivity, the sniffing-licking-gnawing syndrome and the anorexia producted by amphetamine. Since the amount of α-methyl-p-tyrosine which antagonizes the amphetamine stimulation had no effect on the basal level of behavioral performance, Weissman proposed that newly synthesized norepinephrine was essential for the action of amphetamine. However, both amphetamine and 3,4-dihydroxyphenylalanine have recently been shown to reverse the behavioral depression of

a conditioned avoidance response caused by α-methyl-p-tyrosine (Moore and Rech, 1967). These results indicate that the action of amphetamine may be more complex than proposed by Weissman.

Amphetamine has been shown to cause an elevation in the levels of H^3-normetanephrine (Glowinski et al., 1966a), that is, prevent the reuptake of extra-neural norepinephrine by the nerve ending. This study suggests that amphetamine's action consists of blocking the reuptake into the neuron of released norepinephrine.

Relationship Between Catecholamines and 5-Hydroxytryptamine

Vogt (1954) has demonstrated that norepinephrine is distributed in the brain in a manner similar to 5-hydroxytryptamine, being the highest concentration in the brain stem and absent from the cerebellum. The 5-hydroxytryptamine has been shown to release norepinephrine in vitro (Carlsson et al., 1963). Carlsson et al. (1957) also demonstrated that 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine would reverse the sedative effect of reserpine greater than either of the drugs used above. Moreover, Brodie et al. (1966) demonstrated that the intravenous injection of 5-hydroxytryptophan lowered brain norepinephrine levels by 50 per cent in rats and rabbits and elicited increased motor activity, piloerection, and panting. Recently Lal et al. (1969) postulated that 5-hydroxy-

tryptamine, acting through catecholamines, was responsible for amphetamine-induced elevation of the shock level necessary to elicit fighting. These studies suggest the the action of one amine may be mediated by the other amine in the central nervous system.

III. EXPERIMENTAL

Chemicals

Chemicals used were analytical grade or equivalent. Hydroxyamphetamine Sulfate, Dextroamphetamine Sulfate and Chlorpromazine Hydrochloride were obtained through the courtesy of Smith, Kline and French Labs., Philadelphia, Pennsylvania. Reserpine (Serpasil Phosphate) and Syrosingopine were obtained through the courtesy of CIBA Pharmaceutical Company, Summit, New Jersey. Parachlorophenylalanine was obtained through the courtesy of Chas. Pfizer & Co. Inc., Groton, Connecticut. Cyproheptadine was obtained through the courtesy of Merck-Sharp and Dohme Research Laboratories, West Point, Pennsylvania. dl-3,4-dihydroxyphenylalanine (Dopa) was obtained from CALBIOCHEM, Los Angeles, California and from Mann Research Labs., New York, New York. dl-α-methyl-p-tyrosine was obtained from Regis Chemical Company, Chicago, Illinois. The dl-5-hydroxytryptophan was obtained from Aldrich Chemical Company, Cedar Knolls, New Jersey. All drugs were dissolved in distilled water except α-methyl-p-tyrosine and dl-3,4-dihydroxyphenyl-alanine, which was suspended in 0.5 per cent carboxymethyl-cellulose, and parachlorophenylalanine, which was dissolved

according to the procedure of Koe and Weissman (1966).

Animals

Male and female albino rats of Sprague-Dawley strain, random-bred, weighing 200-400 gms., were obtained from Charles River Breeding Farms, Wilmington, Massachusetts. Some of the rats had been used in other behavioral and toxicological experiments prior to their use in this investigation. However, there was at least an interval of one week between other experiments and the training of the animals for this study.

Conditioning and Testing

Training consisted of placing a rat on an electrifiable grid floor of an aluminum chamber (8 in. x 10 in. x 9 in.) containing a wooden platform (4 in. x 6 in. x 2 in.). The conditioned stimulus (CS), a buzzer of fifty-eight decibels, was turned on as soon as the rat was placed in the chamber and then maintained for ten seconds. Responding to the CS with a jump (CR) onto the wooden platform terminated the trial. Failure to respond with a conditioned response resulted in a continuous scrambled foot shock of one milliampere from a Grason-Stadler Shocker, model number E1064GS. The shock was maintained until a jump to escape was made. There was a thirty second intertrial interval. The learning criterion, achieved

in a single sesson, consisted of eight avoidance-responses during ten consecutive trials. Animals which did not reach criterion by thirty trials were not included in the study. After the learning trials, the subjects were returned to home cages for seven days. Testing for retention occurred seven days after the last acquisition trial.

The criterion for learning associated with the drug-state consisted of a significant decrement in responding when the drug or drugs, used in the retention test, produced stimulus-conditions dissimilar to those which occurred during acquisition.

Statistics

All statistical tests, for groups having more than ten rats, were compared using the Chi-square Analysis (Snedecor and Cochran, 1967). Whenever, the group contained less than ten rats, the Exact method of significance (Goldstein, 1967) was used to compare P values.

Unless otherwise stated, a comparison of the per cent avoidance, during performance, to the per cent avoidance of the next to last acquisition trial conducted seven days prior to performance is included in the tables. Wherever appropriate, within-groups comparisons are stated in the text.

IV. RESULTS

Quantitation of an Amphetamine-Produced "Stimulus-State"

In order to determine the lowest dose capable of producing the amphetamine-state, animals were trained with various doses of amphetamine and tested seven days later under the same dose of the drug or under no-drug.

Data summarized in Table I indicate that animals trained under 0 (ND-ND), 0.5 ($A_{.5}$-$A_{.5}$) or 1.0 ($A_{1.0}$-$A_{1.0}$) mg amphetamine per kg body weight showed a significant decrement in avoidance when tested under the same dose of drug. No difference in per cent avoidance during performance is seen if no-drug-trained and no-drug-tested (ND-ND) animals are compared to animals trained and tested under 0.5 mg or 1.0 mg amphetamine per kg body weight (ND-ND vs $A_{0.5}$-$A_{0.5}$ or $A_{1.0}$-$A_{1.0}$, $P > .05$). Animals trained under 2.0 mg amphetamine per kg body weight showed no decrement in avoidance when tested under a similar dose of the drug, ($A_{2.0}$-$A_{2.0}$). However, these animals showed a decrement in avoidance when tested under no-drug, ($A_{2.0}$-ND). No-drug-trained animals showed no decrement in avoidance when tested under 2.0 mg amphetamine per kg body weight, (ND-$A_{2.0}$). Performance of these animals was signifi-

cantly higher than the performance of the animals trained under 2.0 mg amphetamine per kg body weight and tested under no-drug, ($ND\text{-}A_{2.0}$ vs $A_{2.0}\text{-}ND$, $P < .05$).

In order to determine the number of tests which could be performed prior to extinction of avoidance response, amphetamine-trained animals were given repeated tests under amphetamine or no-drug. Similarly, no-drug-trained animals were given repeated tests under no-drug and amphetamine.

Amphetamine-trained animals showed significantly higher avoidance in all four tests than either amphetamine-trained animals tested under no-drug or no-drug-trained animals tested under no-drug, (Figure 1, $A\text{-}A_{1\text{-}4}$ vs $A\text{-}ND_{1\text{-}4}$, $P < .01$; $A\text{-}A_{1\text{-}4}$ vs $ND\text{-}ND_{1\text{-}4}$, $P < .05$). No-drug-trained animals tested under no-drug demonstrated significantly higher avoidance in three of the four tests than amphetamine-trained animals did when tested under no-drug ($ND\text{-}ND_{1,3,4}$ vs $A\text{-}ND_{1,3,4}$, $P < .01$). Testing amphetamine-trained animals under amphetamine, resulted in similar avoidance during the first three tests as did the no-drug-trained animals tested under amphetamine, (Figure 1, $A\text{-}A_{1,2,3}$ vs $ND\text{-}A_{1,2,3}$, $P > .05$). However, during the last test trial the subjects trained and tested under amphetamine showed significantly lower avoidance than those trained under no-drug but tested under amphetamine, ($A\text{-}A_{4}$ vs $ND\text{-}A_{4}$, $P < .05$).

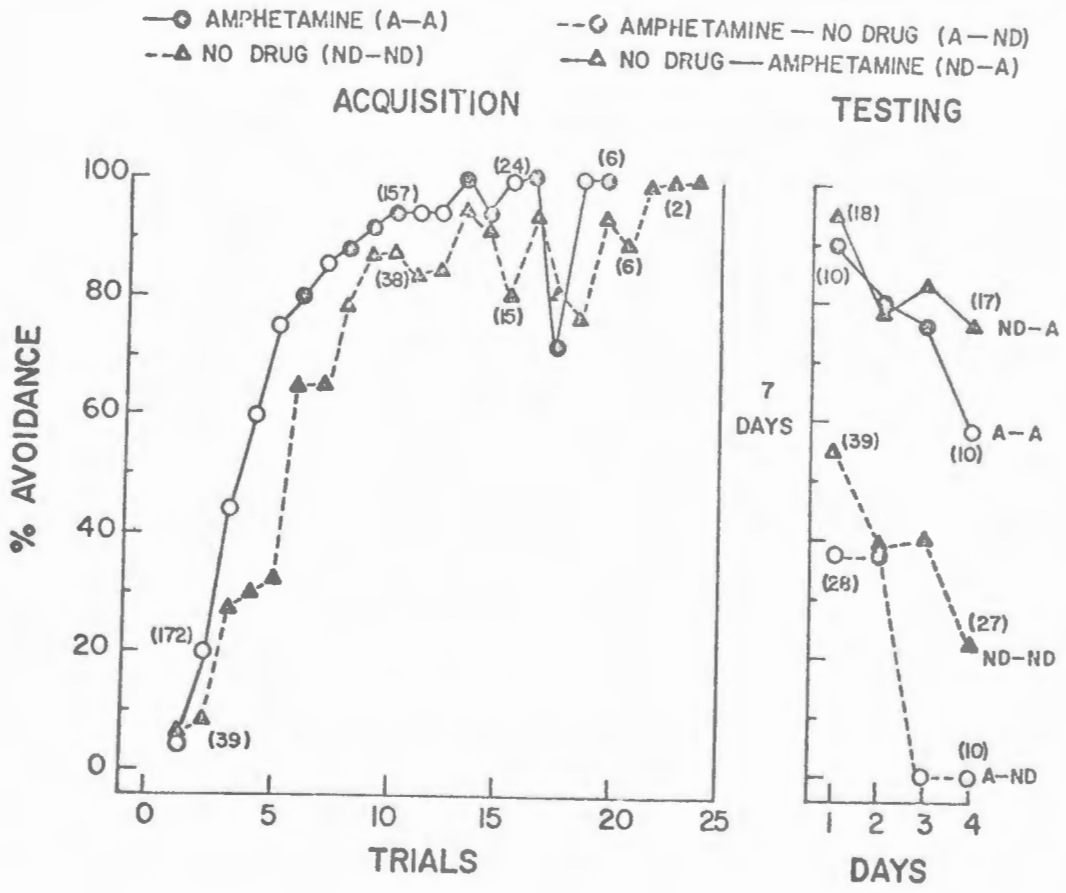

Figure 1 The Effect of Amphetamine on the Acquisition and Performance of a Conditioned Avoidance Response. Open Circles Represent Means Which are Significantly Different (at least at $P < .05$ level) From the Corresponding No-Drug Controls.

TABLE I

EFFECT OF AMPHETAMINE ON PERFORMANCE OF CONDITIONED AVOIDANCE RESPONSES

Group	Drug (mg/kg)[1] Acquisition	Performance	N	% Avoidance	P Acquisition vs Performance
$A_{.5}$-$A_{.5}$	Amphetamine 0.5	Amphetamine 0.5	14	27	$< .01$
$A_{1.0}$-$A_{1.0}$	Amphetamine 1.0	Amphetamine 1.0	15	40	$< .01$[2]
$A_{2.0}$-$A_{2.0}$	Amphetamine 2.0	Amphetamine 2.0	15	80	N.S.
$A_{2.0}$-$A_{1.0}$	Amphetamine 2.0	Amphetamine 1.0	14	27	$< .01$
$A_{2.0}$-ND	Amphetamine 2.0	No Drug —	28	36	$< .05$
ND-$A_{2.0}$	No Drug —	Amphetamine 2.0	10	90	N.S.
ND-ND	No Drug —	No Drug —	39	55	N.S.

[1] 30 min. i.p., prior to performance.

[2] 93% avoidance during last acquisition trial.

Amphetamine-trained animals, which were given four tests under amphetamine (Table II, $A\text{-}A_{1\text{-}4}$) and did not show any decrement in avoidance, showed a significant decrement on the first no-drug test (Table II, $A\text{-}ND_1$). No-drug-trained animals, which showed no decrement in avoidance in any of the four amphetamine tests (Table III, $ND\text{-}A_{1\text{-}4}$), showed a decrement in avoidance on the first no-drug test (Table III, $ND\text{-}ND_1$).

Per cent avoidance of no-drug-trained animals, when tested under amphetamine on the first drug-test, was not significantly different from the performance under amphetamine on the second, third or fourth drug-test ($ND\text{-}A_1$ vs $ND\text{-}A_2$, $ND\text{-}A_3$ or $ND\text{-}A_4$, $P > .05$). Similarly, performance of no-drug-trained animals, tested under no-drug on the first drug-test, did not differ from similarly trained animals, tested under no-drug on the second, third, or fourth test ($ND\text{-}ND_1$ vs $ND\text{-}ND_2$, $ND\text{-}ND_3$ or $ND\text{-}ND_4$, $P > .05$). In addition no significant difference in per cent avoidance is seen when amphetamine-trained animals, tested under amphetamine on the first drug-test, are compared to similarly trained animals tested under amphetamine on the second, third and fourth drug test ($A\text{-}A_1$ vs $A\text{-}A_2$, $A\text{-}A_3$ or $A\text{-}A_4$, $P > .05$). While no further deterioration in performance was seen when amphetamine-trained animals are tested under no-drug on the first and on the second drug-test ($A\text{-}ND_1$ vs

TABLE II

EFFECT OF AMPHETAMINE ON CONSECUTIVE CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER AMPHETAMINE[1]

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
		TEST 1		
$A\text{-}ND_1$	No Drug	28	36	$<.01$
$A\text{-}A_1$	Amphetamine[2]	18	88	N.S.
		TEST 2		
$A\text{-}ND_2$	No Drug	8	37	$<.01$
$A\text{-}A_1$	Amphetamine[2]	18	78	N.S.
		TEST 3		
$A\text{-}ND_3$	No Drug	10	0	$<.01$
$A\text{-}A_3$	Amphetamine[2]	33	76	N.S.
		TEST 4		
$A\text{-}ND_4$	No Drug	14	0	$<.01$
$A\text{-}A_4$	Amphetamine[2]	10	60	N.S.

[1] 2.0 mg/kg, i.p., 30 min prior to acquisition trials.

[2] 2.0 mg/kg, i.p., 30 min prior to performance tests.

TABLE III

EFFECT OF AMPHETAMINE ON CONSECUTIVE CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER NO DRUG

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
		TEST 1		
ND-ND$_1$	No Drug	39	55	$<.05$[2]
ND-A$_1$	Amphetamine[1]	10	90	N.S.
		TEST 2		
ND-ND$_2$	No Drug	19	37	$<.01$[2]
ND-A$_2$	Amphetamine[1]	37	79	N.S.
		TEST 3		
ND-ND$_3$	No Drug	27	41	$<.01$[3]
ND-A$_3$	Amphetamine[1]	17	82	N.S.
		TEST 4		
ND-ND$_4$	No Drug	27	22	$<.01$[3]
ND-A$_4$	Amphetamine	17	78	N.S.

[1] 2.0 mg/kg, i.p., 30 min prior to performance.

[2] 95% avoidance during next to last acquisition trial.

[3] 93% avoidance during next to last acquisition trial.

$A\text{-}ND_2$, $P > .05$), a significant difference was seen when amphetamine-trained animals, tested under no-drug on the first drug-test, are compared to similarly trained animals, tested under no-drug on the third and on the fourth drug tests ($A\text{-}ND_1$ vs $A\text{-}ND_3$, $A\text{-}ND_4$, $P < .05$).

Localization of Stimulus-State

In order to determine whether the amphetamine-state is due to the peripheral or central effects of amphetamine, hydroxyamphetamine was used. Hydroxyamphetamine is an amphetamine-like drug which does not penetrate the central nervous system (Innes and Nickerson, 1965). Data presented in Table IV show that animals trained and tested under 10 mg hydroxyamphetamine per kg body weight showed a decrement in avoidance ($OHA_{10}\text{-}OHA_{10}$). However, testing animals trained under 30 mg hydroxyamphetamine per kg body weight under either a similar dose of the drug or no-drug resulted in no decrement in avoidance. On the other hand, amphetamine-trained animals showed a decrement in avoidance when tested under either dose of hydroxyamphetamine.

Role of Amphetamine's Stimulant Property

If animals, tested under amphetamine, were emitting higher avoidance due to the stimulatory property of amphetamine, then animals trained to a weaker criteria should demonstrate

TABLE IV

EFFECT OF HYDROXYAMPHETAMINE ON CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER HYDROXYAMPHETAMINE OR AMPHETAMINE

Group	Drug (mg/kg)[1] Acquisition		Drug (mg/kg)[1] Performance		N	% Avoidance	P Acquisition vs Performance
A-A	Amphetamine	2	Amphetamine	2	8	88	N.S.
ND-ND	No Drug	-	No Drug	2	39	55	$\angle .05$[2]
$A\text{-}OHA_{10}$	Amphetamine	2	Hydroxy-amphetamine	10	10	20	$\angle .01$
$A\text{-}OHA_{30}$	Amphetamine	2	Hydroxy-amphetamine	30	12	8	$\angle .01$
$OHA_{10}\text{-}OHA_{10}$	Hydroxy-amphetamine	10	Hydroxy-amphetamine	10	12	50	$\angle .05$
$OHA_{30}\text{-}OHA_{30}$	Hydroxy-amphetamine	30	Hydroxy-amphetamine	30	9	88	N.S.
$OHA_{30}\text{-}ND$	Hydroxy-amphetamine	30	No Drug	-	9	66	N.S.

[1] 30 min prior to performance.

[2] 95% avoidance during last acquisition trial.

amphetamine-induced improvement in performance. A significant improvement in per cent avoidance should occur in animals trained to either criteria when no-drug-trained animals are tested under amphetamine. The data summarized in Table V indicate that animals trained to a weaker criteria did not show an improvement in avoidance when tested under amphetamine. Animals trained to the stronger criteria under no-drug showed a decrement in avoidance, when tested under either dl-3,4-dihydroxyphenylalanine or no drug. However, these animals showed no decrement in avoidance when tested under amphetamine. No difference, in per cent avoidance, is seen between animals, trained to the weaker criteria and tested under amphetamine and those tested under no-drug. A significant difference in per cent avoidance is seen between animals, trained to a stronger criteria and tested under amphetamine, and those tested under no-drug or dl-3,4-dihydroxyphenylalanine.

In order to determine if avoidance was due to random jumping off the grid, due to the central nervous system stimulatory effect of amphetamine, random jumping was measured. Data in Table VI show that amphetamine was unable to cause random jumping off the grid.

Drug-Stimulus as a Novel Experience

In order to determine if learning associated with the

TABLE VI

EFFECT OF AMPHETAMINE OF JUMPING OFF THE GRID BY RATS

Treatment	N	% Jumping	P Drug vs Control
No Drug	19	0	N.S.
Saline[1]	9	11	N.S.
Amphetamine[2]	131	3	—

[1] 1 mg/kg, i.p., 30 min prior to trial.

[2] 2.0 mg/kg, i.p., 30 min prior to trial.

amphetamine-state is due to a novel drug-stimulus, amphetamine was injected chronically to a group of rats. These animals, when trained under no-drug, did not show any decrement in per cent avoidance when tested under amphetamine or no-drug (ND_c-A, ND-ND, Table VII). A decrement in avoidance was seen in order chronically treated animals when trained under amphetamine and tested under no-drug (Table VII, A_c-ND). Similarly treated and trained animals showed no decrement in avoidance when tested under amphetamine (A_c-A).

Effect of Selected Drugs on Conditioned Avoidance Response

Since drug-interactions will be used to determine the role of amines in the amphetamine-state, each drug was tested for its acute effects on a newly acquired conditioned avoidance response. Data presented in Table VIII indicate that animals tested under 5-hydroxytryptophan and 4.0 mg chlorpromazine per kg body weight caused a decrement in per cent avoidance of a conditioned avoidance response. Animals tested under reserpine, dl-3,4-dihydroxyphenylalanine, dl-5-hydroxytryptophan and amphetamine also showed a decrement in avoidance of a conditioned avoidance response. Animals tested under amphetamine, reserpine, atropine, chlorpromazine (1.0), syrosingopine, cyproheptadine, hydroxyphenylalanine, or dl-α-methyl-p-tyrosine showed no decrement in per cent avoidance.

TABLE VII

EFFECT OF CHRONIC AMPHETAMINE TREATMENT[1] ON CONDITIONED AVOIDANCE RESPONSES ACQUIRED AND TESTED UNDER AMPHETAMINE[2]

Group	Drug		N	% Avoidance	P
	Acquisition[3]	Performance			Acquisition vs Performance
A_C-A_C	Amphetamine	Amphetamine	18	88	N.S.
A_C-ND_C	Amphetamine	No Drug	18	55	$\leq$.05
ND_C-A_C	No Drug	Amphetamine	16	68	N.S.
ND_C-ND_C	No Drug	No Drug	16	68	N.S.

[1] 7 days prior to acquisition and during the 6 day acquisition-performance interval.

[2] 2.0 mg/kg, i.p., 30 min prior to performance.

[3] Amphetamine not administered 24 h prior to acquisition and prior to performance and 24 h after acquisition.

TABLE VIII

EFFECTS OF VARIOUS DRUGS ON A NEWLY-ACQUIRED CONDITIONED AVOIDANCE RESPONSE

Drug	Dose (mg/kg)	N	% Avoidance	P Acquisition[6] vs Performance
Atropine[1]	5.0	8	75	N.S.
Reserpine[2]	3.0	8	100	N.S.
dl-3,4-dihydroxyphenylalanine[3]	400.0	13	93	N.S.
dl-5-hydroxytryptophan[4]	75.0	12	33	$\angle$.05
Chlorpromazine[3]	1.0	8	100	N.S.
	4.0	10	0	$\angle$.01
Cyproheptadine[5]	10.0	8	100	N.S.
dl-α-Methyl-p-tyrosine[6]	50.0	8	100	N.S.
Syrosingopine[6]	2.5	12	66	N.S.
Hydroxyamphetamine[3]	30.0	8	100	N.S.
Amphetamine[3]	2.0	10	70	N.S.
Reserpine + dl-3,4-dihydroxyphenylalanine + dl-5-hydroxytryptophan + Amphetamine	3.0 + 400.0 + 75.0 + 2.0	8	50	$\angle$.05

TABLE VIII (continued)

[1]45 min prior to performance.

[2]24 h prior to performance.

[3]30 min prior to performance.

[4]1 h prior to performance.

[5]60 min prior to performance.

[6]4 h prior to performance.

Role of Central and Peripheral Amine Stores

If central catecholamines and/or 5-hydroxytryptamine are required for the amphetamine-state to occur, than reserpine, a central and peripheral amine depleting drug (Pletcher et al., 1955; Holzbaurer and Vogt, 1956), should prevent the restoration of the amphetamine-state. Syrosingopine, a peripheral amine depletor (Garrattini, 1959), should cause a decrement in amphetamine state only if peripheral amines are involved in the stimulus-state. Further, if only catecholamines are necessary for the amphetamine-state, then dl-3,4-dihydroxyphenylalanine, a precursor of catecholamines (Gurin and Delluva, 1947), should reverse the effect of reserpine. On the other hand, if 5-hydroxytryptamine is responsible for the amphetamine-state, than dl-5-hydroxytryptophan, a precursor of 5-hydroxytryptamine (Carlesson et al., 1963), should restore the amphetamine effect in reserpinized animals. If both norepinephrine and 5-hydroxytryptamine are needed for the amphetamine-state, then both dl-3,4-dihydroxyphenylalanine and dl-5-hydroxytryptophan will be necessary to alleviate the effect of reserpine.

Data summarized in Table IX indicate that amphetamine-treated animals, when tested under reserpine and amphetamine (A-R+A); or reserpine, dl-3,4-dihydroxyphenylalanine and

amphetamine (A-R+D+A); or reserpine, dl-5-hydroxytryptophan and amphetamine (A-R+5HTP+A), showed a significant decrement in avoidance. However, testing amphetamine-trained animals under reserpine, dl-3,4-dihydroxyphenylalanine, dl-5-hydroxy-tryptophan and amphetamine (A-R+D+5HTP+A) results in no decrement in avoidance. Further, no decrement in per cent avoidance is seen when amphetamine-trained animals are tested under syrosingopine and amphetamine (Table IX, A-S+A). Moreover, a significant difference in per cent avoidance is seen when amphetamine-trained animals tested under syrosingopine and amphetamine or reserpine, dl-3,4-dihydroxyphenylalanine, dl-5-hydroxytryptophan, and amphetamine, are compared to similarly trained animals tested under reserpine and amphetamine (A-R+D+5HTP+A, A-S+A vs A-R+A, $P < .01$); reserpine, dl-5-hydroxytryptophan and amphetamine (A-R+D+5HTP+A, A-S+A vs A-R+5HTP+A, $P < .01$); or reserpine, dl-3,4-dihydroxyphenylalanine and amphetamine (A-R+5HTP+D+A, A-S+A vs A-R+D+A, $P < .01$). No difference in per cent avoidance is seen when amphetamine-trained animals tested under syrosingopine and amphetamine are compared to similarly trained animals tested under reserpine, dl-3,4-dihydroxyphenylalanine, dl-5-hydroxytryptophan and amphetamine (A-S+A vs A-R+D+5HTP+A, $P > .05$). In addition, no significant difference in per cent avoidance occurs between

TABLE IX

EFFECT OF RESERPINE AND SYROSINGOPINE ON CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER AMPHETAMINE

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
A-A	Amphetamine[1]	15	80	N.S.
A-R+A	Reserpine[2] + Amphetamine	8	13	ℓ .01
A-R+D+A	Reserpine + dl-3,4-dihydroxyphenylaline[3] + Amphetamine	16	31	ℓ .01[6]
A-R+5HTP+A	Reserpine + dl-5-hydroxytryptophan[4] + Amphetamine	24	54	ℓ .05[7]
A-R+5HTP+D+A	Reserpine + dl-5-hydroxytryptophan + dl-3,4-dihydroxy-phenylalanine + Amphetamine	15	66	N.S.
A-Syr+A	Syrosingopine[5] + Amphetamine	15	80	N.S.

TABLE IX (continued)

[1]2.0 mg/kg, i.p., 30 min prior to performance.

[2]3.0 mg/kg, i.p., 24 h prior to performance.

[3]400 mg/kg, i.p., 15 min prior to performance.

[4]75 mg/kg, i.p., 1 h prior to performance.

[5]2.5 mg/kg, i.p., 4 h prior to performance.

[6]88% avoidance during last acquisition trial.

[7]96% avoidance during last acquisition trial.

amphetamine-trained animals tested under reserpine, dl-3,4-dihydroxyphenylalanine, dl-5-hydroxytryptophan and amphetamine and no-drug trained animals tested under no-drug (A-R+D+5HTP+A vs ND-ND). The combination of reserpine and dl-3,4-dihydroxyphenylalanine, dl-5-hydroxytryptophan and amphetamine resulted in a significant decrement (Table XII, $P < .05$) in avoidance in a newly acquired conditioned avoidance response.

If a catecholamine, i.e. norepinephrine, released by amphetamine (Carr and Moore, 1969) is responsible for the amphetamine-state, than dl-3,4-dihydroxyphenylalanine should substitute for amphetamine. Similarly, if 5-hydroxytryptamine is responsible for the amphetamine-state, than dl-5-hydroxytryptophan should substitute for amphetamine. Data presented in Table X indicate that, while dl-3,4-dihydroxyphenylalanine substituted for amphetamine (A-D), a significant decrement in per cent avoidance is noted when amphetamine-trained animals are tested under dl-5-hydroxytryptophan, (A-5HTP).

Role of Amine Synthesis

In order to determine if catecholamines, required for amphetamine-state, are newly synthesized or that which is stored, dl-α-methyl-p-tyrosine, an inhibitor of catechol-

amine synthesis (Spector et al., 1956) was used. The time and dose were so selected (Rech et al., 1968) as to allow inhibition of synthesis without depletion of the stored catecholamines. If newly synthesized norepinephrine is needed, than pretreatment under dl-α-methyl-p-tyrosine should cause a decrement in amphetamine-trained animals tested under amphetamine. Replacing the catecholamines by administering dl-3,4-dihydroxyphenylalanine should alleviate this decrement. Similarly, if 5-hydroxytryptamine is involved in the amphetamine-state, than parachlorophenylalanine, an inhibitor of 5-hydroxytryptamine synthesis (Koe and Weissman, 1966), should prevent the amphetamine-state from occuring. Replacing the 5-hydroxytryptamine, by administering dl-5-hydroxytryptophan, should reverse the effect of parachlorophenylalanine.

Data presented in Table X show that pretreatment with dl-α-methyl-p-tyrosine caused a decrement in amphetamine-trained animals when tested under amphetamine (A-MPT+A). The dl-3,4-dihydroxyphenylalanine alleviated the decrement caused by the dl-α-methyl-p-tyrosine. A significant difference in per cent avoidance is seen if amphetamine-trained animals, tested under amphetamine, after pretreatment under dl-α-methyl-p-tyrosine, are compared to similarly trained animals tested under dl-3,4-dihydroxyphenylalanine and amphetamine

after pretreatment under dl-α-methyl-p-tyrosine (A-MPT+A vs A-MPT+D+A, $P < .01$). Moreover, a significant difference ($P < .05$) in per cent avoidance is seen when amphetamine-trained animals were tested under dl-3,4-dihydroxyphenylalanine and compared to amphetamine-trained animals tested under amphetamine and dl-α-methyl-p-tyrosine (A-D vs A-MPT+A, $P < .05$). Performance of amphetamine-trained animals tested under dl-3,4-dihydroxyphenlalanine was not significantly different from similarly trained animals tested under dl-α-methyl-p-tyrosine, dl-3,4-dihydroxyphenlalanine and amphetamine (A-D vs A-MPT+D+A, $P > .05$).

Data in Table X also indicate that parachlorophenylalanine caused a decrement in per cent avoidance in amphetamine-treated animals (A-PCPA+A). However, dl-5 -hydroxytryptophan alleviated the decrement caused by the parachlorophenylalanine (A-PCPA+5HPT+A). Further, a significant difference in per cent avoidance is seen when amphetamine-trained animals were tested under parachlorophenlalanine, dl-5-hydroxytryptophan and amphetamine and were compared to similarly trained animals but tested under either dl-5-hydroxytryptophan alone or parachlorophenylalanine and amphetamine (A-PCPA+5HTP+A vs A-5HTP or A-PCPA+A, $P < .05$).

TABLE X

EFFECT OF P-CHLOROPHENYLALANINE AND DL-α-METHYL-P-TYROSINE ON CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER AMPHETAMINE

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
A-A	Amphetamine[1]	15	80	N.S.
A-MPT+A	dl-α-Methyl-p-tyrosine[2]+ Amphetamine	32	28	$<.01$
A-MPT+D+A	dl-α-Methyl-p-tyrosine + dl-3,4-dihydroxyphenylalanine[3] + Amphetamine	9	78	N.S.
$A\text{-}D_{100}$	dl-3,4-dihydroxyphenylalanine[3]	12	75	N.S.
$A\text{-}D_{400}$	dl-3,4-dihydroxyphenylalanine[4]	13	92	N.S.
A-PCPA+A	Parachlorophenylalanine[5]+ Amphetamine	12	41	$<.05$
A-PCPA+ 5HTP+A	Parachlorophenylalanine + dl-5-Hydroxytryptophan[6] + Amphetamine	12	86	N.S.

TABLE X (continued)

$A\text{-}5HTP_{75}$	dl-5-Hydroxytryptophan[6]	12	25	$< .01$
$A\text{-}5HTP_{150}$	dl-5-Hydroxytryptophan[7]	12	16	$< .01$[8]

[1]2.0 mg/kg, i.p., 30 min prior to performance.

[2]50 mg/kg, i.p., 2 h prior to performance.

[3]100 mg/kg, i.p., 30 min prior to performance.

[4]400 mg/kg, i.p., 30 min prior to performance.

[5]316 mg/kg, i.p., 3 days prior to performance.

[6]75 mg/kg, i.p., 30 min prior to performance.

[7]150 mg/kg, i.p., 30 min prior to performance.

[8]92% avoidance during last acquisition trial.

Amphetamine-State After Inhibition of Catecholamine-Synthesis

If animals are trained and tested under dl-α-methyl-p-tyrosine and amphetamine without showing any decrement in per cent avoidance, than catecholamine synthesis may not be absolutely necessary for the amphetamine-state. Data summarized in Table XI indicate that animals trained under amphetamine after pretreatment under dl-α-methyl-p-tyrosine, when tested under amphetamine after pretreatment under dl-α-methyl-p-tyrosine (MPT+A-MPT+A) or no pretreatment (MPT+A-A) showed no decrement in avoidance. Similarly trained animals, when tested under no-drug (A-MPT-ND), showed a decrement in avoidance. There was a significant difference in per cent avoidance ($P < .01$) between the animals trained and tested under dl-α-methyl-p-tyrosine and amphetamine and the amphetamine-trained animals tested under dl-α-methyl-p-tyrosine and amphetamine (MPT+A-MPT+A vs A-MPT+A).

Role of Amine Receptors

If noradrenergic receptors are involved in the amphetamine-state, than chlorpromazine, a central adrenergic blocking agent (Douglas, 1965), should cause a decrement in amphetamine-state. Similarly, if serotoninergic receptors are involved in the amphetamine-state, than cyproheptadine, a serotoninergic blocking agent (Stone et al., 1961), should

TABLE XI

EFFECT OF AMPHETAMINE AND DL-α-METHYL-P-TYROSINE ON CONDITIONED AVOIDANCE RESPONSES

Group	Acquisition	Performance	N	% Avoidance	P Acquisition vs Performance
A-A	Amphetamine[1]	Amphetamine[1]	10	80	N.S.
MPT+A-MPT+A	dl-α-Methyl-p-tyrosine[2] + Amphetamine[1]	dl-α-Methyl-p-tyrosine[2] + Amphetamine[1]	32	72	N.S.
MPT+A-A	dl-α-Methyl-p-tyrosine[2] + Amphetamine[1]	Amphetamine[1]	17	76	N.S.
MPT+A-ND	dl-α-Methyl-p-tyrosine[2] + Amphetamine[1]	No Drug	9	0	$< .001$
A-MPT+A	Amphetamine[1]	dl-α-Methyl-p-tyrosine[2] + Amphetamine[1]	32	28	$< .01$

[1] 2.0 mg/kg, i.p., 30 min prior to performance.

[2] 50 mg/kg, i.p., 2 h prior to performance.

cause a decrement in amphetamine-state. Data presented in Table XII show that amphetamine-trained animals when tested under amphetamine after pretreatment with chlorpromazine (A-CPZ+A), cyproheptadine (A-Cyp+A), or atropine (A-At+A) exhibited significant decrement in avoidance. Further, a significant difference ($P < .01$) is seen when amphetamine-treated animals tested under amphetamine are compared to similarly trained animals tested under a combination of chlorpromazine and amphetamine ($P < .01$), cyproheptadine and amphetamine ($P < .01$), or atropine and amphetamine ($P < .01$), (A vs CPZ+A, Cyp+A, At+A).

TABLE XII

EFFECT OF CHLORPROMAZINE, CYPROHEPTADINE AND ATROPINE ON CONDITIONED AVOIDANCE RESPONSES ACQUIRED UNDER AMPHETAMINE

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
A-A	Amphetamine[1]	10	80	N.S.
A-CPZ_1+A	Chlorpromazine[2]+ Amphetamine	14	36	$<.01$
A-CPZ_2+A	Chlorpromazine[3]+ Amphetamine	10	20	$<.01$
A-Cyp+A	Cyproheptadine[4]+ Amphetamine	10	30	$<.05$[6]
A-At+A	Atropine[5] + Amphetamine	24	48	$<.01$

[1]2.0 mg/kg, i.p., 30 min prior to performance.

[2]1.0 mg/kg, i.p., 45 min prior to performance.

[3]4.0 mg/kg, i.p., 45 min prior to performance.

[4]10 mg/kg, i.p., 60 min prior to performance.

[5]5.0 mg/kg, i.p., 45 min prior to performance.

[6]90% avoidance during last acquisition trial.

V. DISCUSSION

While animals trained under 0.5 or 1.0 mg amphetamine per kg body weight showed a decrement in avoidance when tested under corresponding doses of amphetamine, animals trained and tested under 2.0 mg amphetamine per kg body weight showed no decrement in avoidance. Using the latter dose of amphetamine, the drug-trained animals showed a decrement in avoidance when tested under no-drug (Figure 1, Table II). Thus amphetamine, in a dose of 2.0 mg/kg, produces a stimulus-state which is capable of controlling avoidance behavior. The animals which are trained under no-drug and tested under no-drug in four consecutive daily tests showed marked decrement in avoidance in all of the tests (ND-ND, Tests 1 to 4, Table III). However, this decrement may have been due to a weaker learning since in a previous study, mice, trained to a stronger criterion under no-drug, showed no decrement in avoidance when tested under no-drug (Lal, 1969).

Since testing of no-drug trained animals under amphetamine resulted in no decrement in avoidance ($ND\text{-}A_{1\text{-}4}$, Table III), then the stimulus-state produced by amphetamine is in only one direction, drug to no-drug. This type of stimulus

control has been called asymetrical dissociation of learning (Overton, 1968). Berger and Stein (1969) explained the asymetry by a neurological model. According to these workers, the brain is chemically differentiated into two hypothetical subsystems; a functionally dominant subsystem that can be affected by the action of drugs and a subordinate subsystem that can resist the action of these drugs. The drug treated animals acquire a task in the subordinate subsystem because the dominant subsystem is blocked by the drug. Testing these animals under no-drug will result in little, if any, demonstration of learned behavior because the dominant subsystem would prevail over the subordinate subsystem which acted as a substitute. Since amphetamine has not been shown to be either an alpha or beta central nervous system blocking agent, this model does not adequately explain the asymetrical dissociation described in this report. The strength of the drug-stimulus may determine whether its stimulus control of behavior is symetrical or asymetrical. Lal (1969) has recently shown that mice, trained under no-drug, showed a decrement in avoidance when tested under chlorpromazine while similarly trained mice showed no decrement in avoidance when tested under amphetamine. However, testing chlorpromazine or amphetamine-trained mice under no-drug, resulted

in a decrement in avoidance. The symetrical dissociation obtained under chlorpormazine may be due to the stronger stimulus-state produced by that drug, while the asymetrical dissociation, obtained under amphetamine, is probably the result of a weak stimulus-state.

The lack of decrement in animals trained under no-drug and tested under amphetamine ($ND\text{-}A_{1\text{-}4}$, Table III) may be interpreted as due to the stimulant property of amphetamine. However, animals tested under a similar dose of drug, but trained to a weaker criteria, also showed a decrement in avoidance (Table V). Thus, it is unlikely that avoidance under amphetamine was only due to the stimulant property of the drug. Moreover, if the avoidance-responses were mere manifestations of locomotor stimulation, then animals treated under amphetamine would show greater number of avoidance responses on the first trial of the acquisition phase. No such first trial avoidances were observed (Table V). Further, if the amphetamine-state did not exist then no difference in per cent avoidance should exist between amphetamine-trained animals tested under no-drug and no-drug trained animals tested under no-drug (A-ND vs ND-ND). On the contrary, there is a significant difference ($P < .05$) in three out of the four tests (Figure 1, $A\text{-}ND_1$ vs $ND\text{-}ND_1$, $A\text{-}ND_3$ vs $ND\text{-}ND_3$,

$A\text{-}ND_4$ vs $ND\text{-}ND_4$). Thus, the stimulant-state, produced by amphetamine, is not the result of the motor stimulant action of the drug.

If amphetamine-associated learning is due to the novelty of action of the drug, then by treating the animals chronically with the drug, this effect should be eliminated. In experiments using chronically treated amphetamine-trained animals, tested under amphetamine, no decrement in avoidance was seen (A_c-A), while similarly trained animals tested under no-drug showed a decrement in avoidance (A_c-ND). In addition, these animals, when trained and tested under no-drug (ND_c-ND), showed no decrement in avoidance (Table VII). The good avoidance seen in the latter group was probably the result of a greater contrast between drug and no-drug states. The strengthened state, which was due to the lack of an amphetamine injection, may have been equivalent to a stronger training criteria. However, the lack of decrement observed when no-drug trained animals were tested under amphetamine could be due to the insufficient stimulus produced by amphetamine.

Animals trained under hydroxyamphetamine, 30 mg/kg, showed no decrement in avoidance when tested under the same dose of that drug (Table IV, OHA_{30}-OHA_{30}). However, this learning is

associated with no apparent drug-state as there was no decrement in response strength when hydroxyamphetamine-trained animals were tested under no-drug (Table IV, OHA_{30}-ND). Since hydroxyamphetamine, which has little central action (Innes and Nickerson, 1969) could not substitute for amphetamine and since reserpine, but not syrosingopine, caused a decrement in amphetamine-trained animals, then the amphetamine-state is central in nature and requires central amines.

Since chlorpromazine, cyproheptadine or atropine caused a decrement in avoidance in amphetamine-treated animals (Table XII, A-CPZ+A, Cyp+A, A-At+A), then the action of catecholamines, 5-hydroxytryptamine or acetylcholine may be involved in the amphetamine-state. However, atropine has been shown to block the increase in free operant avoidance in amphetamine-treated animals (Goldberg and Ciolfolo, 1969). Thus, the decrement in avoidance in the atropinized animals may be the result of a direct depressant effect on the central nervous system by the drug. Therefore, acetylcholine may not be involved in the amphetamine-state.

The dl-3,4-dihydroxyphenylalanine, which alleviated the decrement in avoidance produced by dl-α-methyl-p-tyrosine (Table X, A-MPT+A, A-MPT+D+A) was also found to substitute for amphetamine (Table X, A-D). These results indicate that

catecholamines are involved in the amphetamine-state. However, animals pretreated under dl α-methyl-p-tyrosine and trained under amphetamine showed no decrement in per cent avoidance when tested under either amphetamine or amphetamine after pretreatment under dl-α-methyl-p-tyrosine (MPT+A-A, MPT+A-MPT+A, Table XI). Therefore, animals trained without pretreatment of dl-α-methyl-p-tyrosine are likely to utilize both newly synthesized as well as previously stored catecholamines for release by amphetamine in their stimulus-state. Reduction of the catecholamines synthesis, in these animals, will prevent restoration of this stimulus-state. However, in animals trained with reduced catecholamines synthesis, amphetamine probably utilized previously synthesized catecholamines to produce the stimulus-state. Restoration of the amphetamine-state can occur with either greatly reduced or usual synthesis of catecholamines. Thus, while some released catecholamines seems to be essential for the amphetamine-state, the amount of catecholamines available for release, during training, may determine the amphetamine-state.

The results under 5-hydroxytryptamine seem at first somewhat confusing. The decrement in avoidance when amphetamine-trained animals are tested under amphetamine after parachlorophenylalanine pretreatment indicates that some indolealkyl-

amines are involved in the amphetamine-state. Further, the fact that administered dl-5-hydroxytryptophan, which is taken up by nerve endings (Rodriguez De Lores Arnaiz and De Robertis, 1964) and converted to 5-hydroxytryptamine (Carlsson et al., 1963), alleviated the decrement in avoidance produced by parachlorophenylalanine, also indicated that indolealkylamines are involved in the stimulus-state (Table X, A-PCPA+A, A-PCPA+5HTP+A). In contrast the decrement in per cent avoidance due to substitution of dl-5-hydroxytryptophan for amphetamine (A-5HTP) indicates that indolealkylaminesmay not be involved in the amphetamine-state. However, dl-5-hydroxytryptophan has been demonstrated to depress shock avoidance in rats (Aprison and Hingtgen, 1966). Therefore, excess 5-hydroxy-tryptamine which occurs while testing under dl-5-hydroxy-tryptophan, probably depresses some parts of the central nervous system. The reason that dl-5-hydroxytryptophan is able to alleviate the decrement produced by parachlorophenylalanine is that the latter depresses 5-hydroxytryptamine synthesis so that there is no excess 5-hydroxytryptamine. In addition, if some either substance, i.e., amphetamine, or catecholamines was required for the release of 5-hydroxy-tryptamine, then in the absence of this substance, the 5-hydroxytryptamine would remain within the nerve, and not be

released to produced the amphetamine-state. Since amphetamine has been shown to release 5-hydroxytryptamine only in doses exceeding those used in behavioral studies (Beauvallet et al., 1969), it is unlikely that amphetamine directly releases 5-hydroxytryptamine. Rather, amphetamine probably releases catecholamines (Glowinski et al., 1966b; Carr and Moore, 1969), which in turn releases 5-hydroxytryptamine thereby producing the stimulus-state. These results are consistent with the findings of Lal et al., (1969), in which they hypothesized that 5-hydroxytryptamine acting through catecholamines is responsible for raising the shock level necessary for shock induced fighting.

V. CONCLUSIONS

1) Amphetamine was shown to produce an asymetric stimulus-state which can control conditioned avoidance responses.
2) The amphetamine-state was not based upon a novel drug stimulus.
3) The amphetamine-state depended upon the central catecholamines primarly norepinephrine acting through central serotonin.
4) The level of available catecholamines determined the amphetamine state.
5) Hydroxyamphetamine did not produce a stimulus-state which can control behavior.

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