1970

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

Mark Roffman
University of Rhode Island

Follow this and additional works at: <https://digitalcommons.uri.edu/theses>

Recommended Citation

Roffman, Mark, "Learning Associated with the Amphetamine-State: Role of Brain Amines" (1970). *Open Access Master's Theses*. Paper 220.
<https://digitalcommons.uri.edu/theses/220>

This Thesis is brought to you for free and open access by DigitalCommons@URI. It has been accepted for inclusion in Open Access Master's Theses by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons-group@uri.edu.

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

BY

MARK ROFFMAN

A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
MASTER OF SCIENCE
IN
PHARMACOLOGY AND TOXICOLOGY

UNIVERSITY OF RHODE ISLAND

1970

MASTER OF SCIENCE THESIS

OF

MARK ROFFMAN

Approved:

Thesis Committee:

Chairman

Harold Asch

Nelson F. Smith

James C. F. U.

Dean of the Graduate School

Wm R. Fennell

UNIVERSITY OF RHODE ISLAND

1970

ACKNOWLEDGEMENTS

The author wishes to express his gratitude to his wife, Ina, for her patience, understanding, thoughtfulness and inspiration.

The author wishes to express his gratitude to the members of his graduate committee for their guidance in the preparation of this thesis.

Special thanks are conveyed to Dr. Harbans Lal, his major professor, whose guidance during these experiments and patience with the experimenter were greatly appreciated.

The author also wishes to convey his thanks to his fellow students for their many helpful suggestions during these studies.

The author is very grateful to the University of Rhode Island for providing financial assistance during these studies.

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.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	i
DEDICATION	ii
ABSTRACT.	iii
TABLE OF CONTENTS.	1
LIST OF TABLES.	2
LIST OF FIGURES	3
INTRODUCTION	4
SURVEY OF LITERATURE	6
LEARNING ASSOCIATED WITH A DRUG-STATE.	6
RELATIONSHIP BETWEEN AMPHETAMINE AND NOREPINEPHRINE.	8
RELATIONSHIP BETWEEN CATECHOLAMINES AND 5-HYDROXYTRYPTAMINE	9
EXPERIMENTAL	11
CHEMICALS	11
ANIMALS.	12
CONDITIONING AND TESTING	12
STATISTICS	13
RESULTS	14
DISCUSSION	43
CONCLUSION	51
REFERENCES	52

LIST OF TABLES

Table		Page
I	Effect of amphetamine on performance of conditioned avoidance responses	17
II	Effect of amphetamine on consecutive conditioned avoidance responses acquired under amphetamine	19
III	Effect of amphetamine on consecutive conditioned avoidance responses acquired under no-drug	20
IV	Effect of hydroxyamphetamine on conditioned avoidance responses acquired under hydroxyamphetamine or amphetamine.	22
V	Effect of amphetamine on conditioned avoidance responses acquired to different criteria under no-drug	24
VI	Effect of amphetamine on jumping off the grid by rats.	26
VII	Effect of chronic amphetamine treatment on conditioned avoidance responses acquired and tested under amphetamine	27
VIII	Effects of various drugs on a newly-acquired conditioned avoidance response	28
IX	Effect of reserpine and syrosingopine on conditioned avoidance responses acquired under amphetamine	32
X	Effect of p-chlorophenylalanine and dl- L -methyl-p-tyrosine on conditioned avoidance responses acquired under amphetamine	37
XI	Effect of amphetamine and dl- L -methyl-p-tyrosine on conditioned avoidance responses.	40
XII	Effect of chlorpromazine, cyproheptadine, and atropine on conditioned avoidance responses acquired under amphetamine	42

LIST OF FIGURES

Figure	Page
1 The Effect of Amphetamine on Acquisition and Performance of Conditioned Avoidance Response. Open Circles Represent Means Which are Significantly Different (at least at $P < .05$ level) From the Corresponding No-Drug Controls	16

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 produced 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-dihydroxyphenylalanine, 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 milliamperes 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 session, 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, ($ND-A_{2.0}$ vs $A_{2.0}-ND$, $P < .05$).

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, $A-A_{1-4}$ vs $A-ND_{1-4}$, $P < .01$; $A-A_{1-4}$ vs $ND-ND_{1-4}$, $P < .05$). 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 ($ND-ND_{1,3,4}$ vs $A-ND_{1,3,4}$, $P < .01$). 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, $A-A_{1,2,3}$ vs $ND-A_{1,2,3}$, $P > .05$). 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, ($A-A_4$ vs $ND-A_4$, $P < .05$).

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 $P \leq .05$ level) From the Corresponding No-Drug Controls.

TABLE I
EFFECT OF AMPHETAMINE ON PERFORMANCE OF CONDITIONED AVOIDANCE RESPONSES

Group	Drug (mg/kg) ¹		N	% Avoidance	P
	Acquisition	Performance			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.

¹30 min. i.p., prior to performance.

²93% avoidance during last acquisition trial.

Amphetamine-trained animals, which were given four tests under amphetamine (Table II, A-A₁₋₄) and did not show any decrement in avoidance, showed a significant decrement on the first no-drug test (Table II, A-ND₁). No-drug-trained animals, which showed no decrement in avoidance in any of the four amphetamine tests (Table III, ND-A₁₋₄), showed a decrement in avoidance on the first no-drug test (Table III, ND-ND₁).

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-A₁ vs ND-A₂, ND-A₃ or ND-A₄, $P > .05$). 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-ND₁ vs ND-ND₂, ND-ND₃ or ND-ND₄, $P > .05$). 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-A₁ vs A-A₂, A-A₃ or A-A₄, $P > .05$). 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-ND₁ vs

TABLE II
EFFECT OF AMPHETAMINE ON CONSECUTIVE CONDITIONED AVOIDANCE
RESPONSES ACQUIRED UNDER AMPHETAMINE¹

Group	Treatment Prior to Performance	N	% Avoidance	P Acquisition vs Performance
TEST 1				
A-ND ₁	No Drug	28	36	4.01
A-A ₁	Amphetamine ²	18	88	N.S.
TEST 2				
A-ND ₂	No Drug	8	37	4.01
A-A ₁	Amphetamine ²	18	78	N.S.
TEST 3				
A-ND ₃	No Drug	10	0	4.01
A-A ₃	Amphetamine ²	33	76	N.S.
TEST 4				
A-ND ₄	No Drug	14	0	4.01
A-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	4.05 ²
ND-A ₁	Amphetamine ¹	10	90	N.S.
TEST 2				
ND-ND ₂	No Drug	19	37	4.01 ²
ND-A ₂	Amphetamine ¹	37	79	N.S.
TEST 3				
ND-ND ₃	No Drug	27	41	4.01 ³
ND-A ₃	Amphetamine ¹	17	82	N.S.
TEST 4				
ND-ND ₄	No Drug	27	22	4.01 ³
ND-A ₄	Amphetamine	17	78	N.S.

¹2.0 mg/kg, i.p., 30 min prior to performance.

²95% avoidance during next to last acquisition trial.

³93% avoidance during next to last acquisition trial.

A-ND₂, $P > .05$), 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-ND₁ vs A-ND₃, A-ND₄, $P < .05$).

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 ($OHA_{10} - OHA_{10}$). 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				N	% Avoidance	P Acquisition vs Performance
	Acquisition		Performance				
A-A	Amphetamine	2	Amphetamine	2	8	88	N.S.
ND-ND	No Drug	-	No Drug	2	39	55	$\angle .05^2$
A-OHA ₁₀	Amphetamine	2	Hydroxy- amphetamine	10	10	20	$\angle .01$
A-OHA ₃₀	Amphetamine	2	Hydroxy- amphetamine	30	12	8	$\angle .01$
OHA ₁₀ - OHA ₁₀	Hydroxy- amphetamine	10	Hydroxy- amphetamine	10	12	50	$\angle .05$
OHA ₃₀ - OHA ₃₀	Hydroxy- amphetamine	30	Hydroxy- amphetamine	30	9	88	N.S.
OHA ₃₀ - ND	Hydroxy- amphetamine	30	No Drug	-	9	66	N.S.

¹30 min prior to performance.

²95% 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 TREATMENT¹ ON CONDITIONED AVOIDANCE
RESPONSES ACQUIRED AND TESTED UNDER AMPHETAMINE²

Group	Drug		N	% Avoidance	P Acquisition vs Performance
	Acquisition ³	Performance			
A _c -A _c	Amphetamine	Amphetamine	18	88	N.S.
A _c -ND _c	Amphetamine	No Drug	18	55	4.05
ND _c -A _c	No Drug	Amphetamine	16	68	N.S.
ND _c -ND _c	No Drug	No Drug	16	68	N.S.

¹7 days prior to acquisition and during the 6 day acquisition-performance interval.

²2.0 mg/kg, i.p., 30 min prior to performance.

³Amphetamine 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	Acquisition ⁶ vs Performance ^P
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	2.05
Chlorpromazine ³	1.0	8	100	N.S.
	4.0	10	0	2.01
Cyproheptadine ⁵	10.0	8	100	N.S.
dl-2-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 +	3.0			
dl-3,4-dihydroxyphenylalanine +	400.0	8	50	2.05
dl-5-hydroxytryptophan +	75.0			
Amphetamine	2.0			

TABLE VIII (continued)

¹45 min prior to performance.

²24 h prior to performance.

³30 min prior to performance.

⁴1 h prior to performance.

⁵60 min prior to performance.

⁶4 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 (Carlessen 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-hydroxytryptophan 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 SYROSIINGOPINE 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	$\leq .01$
A-R+D+A	Reserpine + dl-3,4- dihydroxyphenylalanine ³ + Amphetamine	16	31	$\leq .01$ ⁶
A-R+5HTP+A	Reserpine + dl-5- hydroxytryptophan ⁴ + Amphetamine	24	54	$\leq .05$ 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)

¹2.0 mg/kg, i.p., 30 min prior to performance.

²3.0 mg/kg, i.p., 24 h prior to performance.

³400 mg/kg, i.p., 15 min prior to performance.

⁴75 mg/kg, i.p., 1 h prior to performance.

⁵2.5 mg/kg, i.p., 4 h prior to performance.

⁶88% avoidance during last acquisition trial.

⁷96% 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 occurring. 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-dihydroxyphenylalanine was not significantly different from similarly trained animals tested under dl- α -methyl-p-tyrosine, dl-3,4-dihydroxyphenylalanine 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 parachlorophenylalanine, 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+5HPT+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-dihydroxyphenylal- anine ³ + Amphetamine	9	78	N.S.
A-D ₁₀₀	dl-3,4-dihydroxyphenylal- anine ³	12	75	N.S.
A-D ₄₀₀	dl-3,4-dihydroxyphenylal- anine 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-5HTP ₇₅	dl-5-Hydroxytryptophan ⁶	12	25	< .01
A-5HTP ₁₅₀	dl-5-Hydroxytryptophan ⁷	12	16	< .01 ⁸

¹2.0 mg/kg, i.p., 30 min prior to performance.

²50 mg/kg, i.p., 2 h prior to performance.

³100 mg/kg, i.p., 30 min prior to performance.

⁴400 mg/kg, i.p., 30 min prior to performance.

⁵316 mg/kg, i.p., 3 days prior to performance.

⁶75 mg/kg, i.p., 30 min prior to performance.

⁷150 mg/kg, i.p., 30 min prior to performance.

⁸92% 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 serotonergic receptors are involved in the amphetamine-state, than cyproheptadine, a serotonergic 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

¹2.0 mg/kg, i.p., 30 min prior to performance.

²50 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

¹2.0 mg/kg, i.p., 30 min prior to performance.

²1.0 mg/kg, i.p., 45 min prior to performance.

³4.0 mg/kg, i.p., 45 min prior to performance.

⁴10 mg/kg, i.p., 60 min prior to performance.

⁵5.0 mg/kg, i.p., 45 min prior to performance.

⁶90% 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-A₁₋₄, 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 asymmetrical dissociation of learning (Overton, 1968). Berger and Stein (1969) explained the asymmetry 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 asymmetrical dissociation described in this report. The strength of the drug-stimulus may determine whether its stimulus control of behavior is symmetrical or asymmetrical. 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 symmetrical dissociation obtained under chlorpormazine may be due to the stronger stimulus-state produced by that drug, while the asymmetrical 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-A₁₋₄, 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-ND₁ vs ND-ND₁, A-ND₃ vs ND-ND₃,

A-ND₄ vs ND-ND₄). 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, OHA₃₀-OHA₃₀). 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, OHA₃₀-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 Ciofalo, 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 indolealkylamines may 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-hydroxytryptamine which occurs while testing under dl-5-hydroxytryptophan, 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-hydroxytryptamine, 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 asymmetric 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 primarily 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.

REFERENCES

- Aprison, M. H. and Hingtgen, J. N.: Neurochemical correlates of behavior. V. Differential effects of drugs on approach and avoidance behavior in rats with related changes in brain serotonin and norepinephrine. Recent Advances in Biological Psychiatry 8: 87-99, 1966.
- Ashford, A., Penn, G. B. and Ross, J.: Cholinergic activity of atropine. Nature 193: 1082-1085, 1962.
- Beauvallet, M., Weil-Fugazza, J., Legrand, M. and Soliek, M.: Amphetamine and brain 5-hydroxytryptophan. Abstracts Intern. Cong. Pharmacol. 4: 64, 1969.
- Belleville, R. E.: Control of behavior by drug produced internal stimuli. Psychopharmacologia (Berl.) 5: 95-104, 1964.
- Berger, B. and Stein, L.: Asymmetrical dissociation of learning between scopolamine and WY-4036, a new benzodiazepine tranquilizer. Psychopharmacologia 14: 351-358, 1969.
- Brodie, B. B., Comer, M. S., Costa E. and Dlabac, A.: The role of serotonin in the mechanism of the central action of reserpine. J. Pharmacol. Exp. Therap. 152: 340-359, 1966.
- Carlsson, A., Hillarp, N. A. and Waldeck, B.: Analysis of Mg^{++} -ATP dependent mechanism in the amine granules of the adrenal medulla. Acta Physiol. Scand. 59: supp. 215, 138, 1963.
- Carlsson, A., Lindquist, M. and Magnusson, T.: 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. Nature 180: 1200, 1957.
- Carr, L. A. and Moore, K. E.: Norepinephrine release from brain by d-amphetamine in vivo. Science 164: 322-323, 1969.
- Douglas, W. W.: 5-Hydroxytryptamine and antagonists: Polypeptides-angiotensin and kinine. In the Pharmacol. Basis of Therap., ed. by L. S. Goodman and A. Gilman, pp. 644-663, MacMillan Co., New York, 1965.

- Garrattini, S.: Reserpine derivative with specific hypotensive sedative activity. *Nature* 183: 1273-1274, 1959.
- Glowinski, J., Axelrod, J. and Iverson, L.: Regional studies of catecholamines in the rat brain, IV. Effects of drugs on the disposition and metabolism of H^3 -norepinephrine and H^3 -dopamine. *J. Pharmacol. Exp. Therap.* 153: 30-41, 1966A.
- Glowinski, J., Snyder, S. H. and Axelrod, J.: Subcellular localization of H^3 -norepinephrine in rat brain and the effects of drugs. *J. Pharmacol. Exp. Therap.* 152: 282-292, 1966B.
- Goldberg, M. E., and Ciofalo, V. B.: Alteration of the behavioral effects of amphetamine by agents which modify cholinergic function. *Psychopharmacologia (Berl.)* 14: 142-149, 1969.
- Goldstein, A.: *Biostatistics: An Introductory Text.* MacMillan Co., pp. 111-112, 1967.
- Gurin, S. and Delluva, A.: The biological synthesis of radioactive adrenalin from phenylalanine. *J. Biol. Chem.* 170: 545-550, 1947.
- Harvey, S. C., Sulkowski, T. S. and Weenig, D. J.: Effect of amphetamines on plasma catecholamines. *Arch. Intern. Pharmacodyn.* 172: 301-322, 1968.
- Holzbaurer, M., and Vogt, M.: Depression by reserpine of the noradrenaline concentration in the hypothalamus of the cat. *J. Neurochem.* 1: 8-11, 1956.
- Innes, I. R., and Nickerson, M.: Drugs acting on postganglionic adrenergic nerve endings and structures innervated by them. In *the Pharmacol. Basis of Therap.*, ed. by L. S. Goodman and A. Gilman, pp. 500-513, MacMillan Co., New York, 1965.
- Kubena, R. and Barry, H.: Generalization by rats of alcohol and atropine stimulus characteristics to other drugs. *Psychopharmacologia* 15: 196-206, 1969.

- Koe, K. and Weissman, A.: P-chlorophenylalanine: A specific depletor of brain serotonin. *J. Pharmacol. Exp. Therap.* 154: 499-516, 1966.
- Lal, H.: Control of learned conditioned-avoidance responses (CAR) by amphetamine and chlorpromazine. *Psychopharmacologia (Berl.)* 14: 33-37, 1969.
- Lal, H., DeFeo, J. and Thut, P.: Amphetamine-induced blockade of experimental aggression: Possible involvement of catecholamines as well as indole-alkylamine. *The Pharmacologist.* 11: 278, 1969.
- Moore, K. E. and Rech, R. H.: Reversal of alpha-methyl-tyrosine-induced behavioral depression with dihydroxy-phenylalanine and amphetamine. *J. Pharm. Pharmacol.* 19: 405-407, 1967.
- Otis, L. S.: Dissociation and recovery of a response learned under the influence of chlorpromazine or saline. *Science* 143: 1347-1348, 1964.
- Overton, D. A.: State-dependent or "dissociated" learning produced with pentobarbital. *J. Comp. Physiol. Psychol.* 57: 3-12, 1964.
- Overton, D. A.: State-dependent learning produced by depressant and atropine-like drugs. *Psychopharmacologia (Berl.)* 10: 6-31, 1966.
- Overton, D. A.: Dissociated learning in the drug states. *Psychopharmacology. A Review of Progress, 1957-1967*, ed. by D. H. Efron, U. S. Gov't. Printing Office, pp. 918-927, 1968.
- Pletscher, A., Shore, P. A. and Brodie, B. B.: Serotonin release as a possible mechanism of reserpine action. *Science* 122: 374-375, 1955.
- Rech, R. H., Carr, A. and Moore, K. E.: Behavioral effects of -methyltyrosine after prior depletion of brain catecholamines. 160: 326-359, 1968.
- Rodriguez De Lores Arnaiz, R. and DeRobertis, L.: 5-Hydroxytryptophan decarboxylase activity in nerve endings in the rat brain. *J. Neurochem.* 11: 213-219, 1964.

- Roffman, M. and Lal, H.: Effect of amphetamine on effectiveness of extinction procedure to decelerate operant behavior. *The Pharmacologist* 11: 246, 1969.
- Snedecor, G. W. and Cochran, W. B.: Statistical methods. The Iowa State Univ. Press, pp. 228-240, 1967.
- Spector, S., Sjoerdsma, A. and Udenfriend, S.: Blockade of endogenous norepinephrine synthesis by alpha-methyl-tyrosine, an inhibitor of tyrosine hydroxylase. *J. Pharmacol. Exp. Therap.* 147: 86-102, 1965.
- Stone, C. A., Wenger, H. C., Ludden, C. T., Stavorski, J. M. and Ross, C. A.: Antiserotonin-antihistamine properties of cyproheptadine. *J. Pharmacol. Exp. Therap.* 131: 73-84, 1961.
- Udenfriend, S.: 5-Hydroxytryptamine. Ed. by G. P. Lewis, pp. 14-34, Pergamon Press, 1959.
- Vogt, M.: The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. *J. Physiol.* 123: 451-458, 1954.
- Weissman, A., Koe, K. and Tenen, S. S.: Antiamphetamine effects following inhibition of tyrosine hydroxylase. *J. Pharmacol. Exp. Therap.* 151: 339-352, 1966.
- Welch, B. and Welch, A.: Stimulus-dependent antagonism of the alpha-methyl-tyrosine-induced lowering of brain catecholamines by d-amphetamine in intact mice. *J. Pharm. Pharmacol.* 19: 842-843, 1967.