1980

Stress, Neurotransmitters and Avoidance Acquisition

Daniel Leo Richardson
Loyola University Chicago

Recommended Citation
Richardson, Daniel Leo, "Stress, Neurotransmitters and Avoidance Acquisition" (1980). Dissertations. 1849.
https://ecommons.luc.edu/luc_diss/1849

This Dissertation is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Dissertations by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.
Creative Commons License
This work is licensed under a Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License.
Copyright © 1980 Daniel Leo Richardson
STRESS, NEUROTRANSMITTERS
AND AVOIDANCE ACQUISITION

BY

Daniel L. Richardson

A Dissertation Submitted to the Faculty of the
Department of Pharmacology
Loyola University Medical Center
in Partial Fulfillment of the Requirements for the
Degree of Doctor of Philosophy
ACKNOWLEDGEMENTS

Drs. Alexander G. Karczmar and Charles L. Scudder provided the framework and financial support for this work. Dr. Stanley A. Lorens played a major role with his encouragement and criticism. Drs. Michael A. Collins, Silas N. Glisson, Alfred J. Kahn and R. Alan North are to be thanked for their interest and perseverance. Mrs. Stanley A. Lorens donated her considerable typing skills. The technical assistance and persistent support from Ms. Gisela Kindel is gratefully acknowledged.
VITA

The author, Daniel Leo Richardson, is the son of Leo and Arlene Richardson. He was born October 30, 1942 in Rockford Illinois.

His elementary education was obtained in the parochial schools of Rockford and secondary education at the St. Thomas High School, Rockford, where he graduated in 1961.

In 1961 he entered Loyola University, Chicago, and in 1965 received the degree of Bachelor of Science.

In 1967 he entered the Loyola University Graduate School, Department of Pharmacology, and was awarded a Master of Science degree in 1972.

In 1972 he joined the faculty at Rockford School of Medicine as an Assistant Professor of Pharmacology. In 1975 he returned to Loyola University to complete the work on his doctorate degree.

The following is a list of his publications:


Richardson, D. L. Correlations between behavioral changes and drug induced variations of levels of mouse brain CA and 5-HT. Masters Thesis, Loyola University Medical Center, Dept. Pharm. 1972.


## TABLE OF CONTENTS

ACKNOWLEDGEMENTS ........................................... ii
VITA .......................................................... iii
LIST OF ABBREVIATIONS ................................................ iv
LIST OF TABLES ......................................................... vi
LIST OF ILLUSTRATIONS ................................................ vii
CHAPTER I. OBJECTIVES ................................................. 1

CHAPTER II. METHODS AND MATERIALS ................................. 7

A. ANIMALS ............................................................ 7

B. BEHAVIORAL MEASURES ........................................... 7

1. Avoidance Conditioning "Climbing Screen" ......................... 7

2. Locomotor Activity "Photoactometers" ............................. 12

3. Aggression (Isolation Induced) ..................................... 12

4. Foot Shock ....................................................... 14

C. BRAIN EXTRACTION AND DISSECTION ............................. 15

D. TEMPERATURE MEASUREMENTS ..................................... 17

E. SURGICAL PROCEDURES ........................................... 17

1. Glossopharyngealectomy .......................................... 17

2. Enucleation ..................................................... 18

3. Olfactory Bulbectomy ............................................. 18

F. BIOCHEMICAL DETERMINATIONS ................................... 19

1. Norepinephrine (NE) and Serotonin (5-HT) Concentrations .......... 19

2. Gamma-Amino-Butyric-Acid (GABA) Level .......................... 22

3. Acetylcholine (ACh) Level ....................................... 25
TABLE OF CONTENTS (CON'T)

4. 5-hydroxyindole Acetic Acid (5-HIAA)
   Level ........................................ 29

5. ACh Turnover. ............................... 30

6. NE Turnover ................................. 32

G. STATISTICAL ANALYSIS ..................... 34

CHAPTER III. CHANGES IN CENTRAL TRANSMITTER LEVELS
AND TURNOVER ASSOCIATED WITH STRESS. .......... 35

A. INTRODUCTION ............................... 35

B. EXPERIMENTAL DESIGN ....................... 43
   1. Effect of Foot Shock Stress on Brain NE,
      5-HT, ACh and GABA Levels. ............... 43
   2. Time Course of the Effects of Foot
      Shock Stress on Brain NE and
      5-HT Levels .............................. 49
   3. Effect of Foot Shock Stress on NE
      Concentration in Brain Parts, and
      on Whole Brain NE and ACh Turnover
      and 5-HIAA Level ......................... 49
      a. Brain Parts .......................... 49
      b. Whole Brain ACh and NE Turnover
         Rate .................................. 50
      c. Whole Brain 5-HIAA Level .......... 50

C. RESULTS .................................... 50

D. DISCUSSION ................................. 51

CHAPTER IV. LEARNING AND NEUROCHEMICAL VARIABLES. ..... 65

A. INTRODUCTION ............................... 65

B. EXPERIMENTAL DESIGN ....................... 67
   Effect of Acquisition of a Conditioned
   Avoidance Response (CAR) on NE, 5-HT and
   ACh Levels ............................... 67
### TABLE OF CONTENTS (CON'T)

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. RESULTS</td>
<td>69</td>
</tr>
<tr>
<td>D. DISCUSSION</td>
<td>70</td>
</tr>
</tbody>
</table>

**CHAPTER V. LEARNING AND STRESS.** 78

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. INTRODUCTION</td>
<td>78</td>
</tr>
<tr>
<td>B. EXPERIMENTAL DESIGN</td>
<td>79</td>
</tr>
<tr>
<td>1. Effects of Stress on Acquisition of a Conditioned Avoidance Response (CAR)</td>
<td>79</td>
</tr>
<tr>
<td>2. Effects of CAR Acquisition on Stress Induced Changes in Brain Transmitter Levels</td>
<td>80</td>
</tr>
<tr>
<td>C. RESULTS</td>
<td>81</td>
</tr>
<tr>
<td>D. DISCUSSION</td>
<td>84</td>
</tr>
</tbody>
</table>

**CHAPTER VI. DRUGS, NEUROCHEMISTRY AND LEARNING.** 87

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. INTRODUCTION</td>
<td>87</td>
</tr>
<tr>
<td>1. Catecholamine Systems</td>
<td>87</td>
</tr>
<tr>
<td>2. Cholinergic Systems</td>
<td>90</td>
</tr>
<tr>
<td>3. Serotonergic Systems</td>
<td>91</td>
</tr>
<tr>
<td>4. Summary</td>
<td>91</td>
</tr>
<tr>
<td>B. EXPERIMENTAL DESIGN</td>
<td>92</td>
</tr>
<tr>
<td>Effects of Neurotransmitter Modifying Drugs on the Acquisition of a CAR, Neurochemistry and Activity</td>
<td>92</td>
</tr>
<tr>
<td>C. RESULTS</td>
<td>94</td>
</tr>
<tr>
<td>D. DISCUSSION</td>
<td>97</td>
</tr>
</tbody>
</table>

**CHAPTER VII. MOUSE STRAINS, NEUROCHEMISTRY AND LEARNING.** 100

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. INTRODUCTION</td>
<td>100</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS (CON'T)

B. EXPERIMENTAL DESIGN. .................................. 102

Mouse Strain Differences in Neurochemistry, Acquisition of a CAR and Activity. ........ 102

1. Neurochemical Studies .................................. 102

2. Behavioral Studies ...................................... 102

C. RESULTS. .................................................. 102

1. NE, 5-HT, ACh and GABA Levels in Whole Brain. ........ 102

2. Avoidance Conditioning .................................. 102

3. Locomotor Activity ...................................... 104

D. DISCUSSION ................................................ 104

CHAPTER VIII. NEUROCHEMICAL AND BEHAVIORAL EFFECTS OF SENSORY DEPRIVATION OR STRESSORS OTHER THAN FOOT SHOCK. ......................... 107

A. INTRODUCTION ............................................ 107

B. EXPERIMENTAL DESIGN. .................................. 111

1. Glossopharyngealotomy .................................. 111

2. Enucleation .............................................. 111

3. Olfactory Bulbectomy .................................... 112

4. Isolation ................................................ 113

C. RESULTS .................................................... 114

1. Biochemistry ............................................ 114

2. Avoidance Conditioning ................................ 114

3. Locomotor Activity ..................................... 114

4. Aggression ................................................ 114

D. DISCUSSION ................................................ 118
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAPTER IX. SUMMARY, CONCLUSIONS AND</td>
<td>120</td>
</tr>
<tr>
<td>SPECULATIONS</td>
<td></td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>124</td>
</tr>
</tbody>
</table>
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenal Corticotropic Hormone</td>
</tr>
<tr>
<td>AKG</td>
<td>Alpha-Ketoglutarate</td>
</tr>
<tr>
<td>α-MPT</td>
<td>Alpha-methyl-para-tyrosine</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>C</td>
<td>Centigrade</td>
</tr>
<tr>
<td>CA</td>
<td>Catecholamines</td>
</tr>
<tr>
<td>CAR</td>
<td>Conditioned Avoidance Response</td>
</tr>
<tr>
<td>Cere</td>
<td>Cerebellum</td>
</tr>
<tr>
<td>C57BL/By</td>
<td>Mus Musculus C57BL/Bailey</td>
</tr>
<tr>
<td>CF-1</td>
<td>Mus Musculus CF-1</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>L-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenedinitrilo tetra acetic acid</td>
</tr>
<tr>
<td>F</td>
<td>Farenheit</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>5-HIAA</td>
<td>L-5-hydroxyindole acetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>L-5-hydroxytryptamine=Serotonin</td>
</tr>
<tr>
<td>5-HTP</td>
<td>L-5-hydroxytryptophan</td>
</tr>
<tr>
<td>³H</td>
<td>Tritiated hydrogen atom</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>H₂O</td>
<td>Water</td>
</tr>
<tr>
<td>hR</td>
<td>Hour</td>
</tr>
<tr>
<td>in.</td>
<td>Inch</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneally</td>
</tr>
<tr>
<td>Kg</td>
<td>Kilogram</td>
</tr>
<tr>
<td>KPPO₄</td>
<td>Potassium Pyrophosphate</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>MD</td>
<td>Midbrain-Diencephalon</td>
</tr>
<tr>
<td>μg</td>
<td>microgram</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>µl</td>
<td>Microliter</td>
</tr>
<tr>
<td>ml</td>
<td>Milliter</td>
</tr>
<tr>
<td>mm</td>
<td>Millimeter</td>
</tr>
<tr>
<td>N</td>
<td>Normal</td>
</tr>
<tr>
<td>NADP</td>
<td>Nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>Sodium Bicarbonate</td>
</tr>
<tr>
<td>Na₂SO₃</td>
<td>Sodium Thiosulfite</td>
</tr>
<tr>
<td>nM</td>
<td>nanomole</td>
</tr>
<tr>
<td>³²P</td>
<td>Phosphorus isotope 32</td>
</tr>
<tr>
<td>pCPA</td>
<td>Para-chlorophenylalanine</td>
</tr>
<tr>
<td>PM</td>
<td>Pons-Medulla</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid Eye Movement Sleep</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>sec</td>
<td>Second</td>
</tr>
<tr>
<td>T</td>
<td>Telencephalon</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume by volume</td>
</tr>
</tbody>
</table>
LIST OF TABLES

I. Stress and Neurotransmitters: Summary of the Literature. ........... 44

II. Effect of Stress on Norepinephrine Level in Brain Parts, Acetylcholine and Norepinephrine Turnover Rate in Whole Brain, and 5-hydroxyindole-Acetic-Acid Level in Whole Brain .... 58

III. Drugs, Mechanism of Action, Action on Neurotransmitter and Dose Schedule. ........ 93

IV. Effect of Drugs on Whole Brain Neurotransmitters, Avoidance Conditioning and Activity. ............ 96

V. Endogenous Whole Brain Concentrations of Norepinephrine, 5-hydroxytryptamine, Acetylcholine and Gamma-Aminobutyric-Acid in Two Strains of Mus .......... 103

VI. Lack of Effect of Four Stressors on Whole Brain Norepinephrine and 5-hydroxytryptamine Concentrations in CF-1 Mice ............. 116
LIST OF ILLUSTRATIONS

1. Photograph of the Automated Avoidance Conditioning "Climbing Screen" Apparatus ............. 8
2. Diagram of the Automated Avoidance Conditioning "Climbing Screen". .................. 9
3. Effect of Consecutive Foot Shocks on Whole Brain Norepinephrine Level ............... 52
4. Effect of Consecutive Foot Shocks on Whole Brain 5-hydroxytryptamine Level ......... 53
5. Effect of Consecutive Foot Shocks on Whole Brain Acetylcholine Level ................. 54
6. Effect of Consecutive Foot Shocks on Whole Brain Gamma-Aminobutyric-Acid Level ...... 55
7. Time Course for the Normalization of Whole Brain Norepinephrine Level After Cessation of Foot Shock Stress ............................ 56
8. Time Course for the Normalization of Whole Brain 5-hydroxytryptamine Level After Cessation of Foot Shock Stress ............... 57
9. Effect of Avoidance Conditioning and Foot Shock Stress on Whole Brain 5-hydroxytryptamine, Norepinephrine and Acetylcholine Levels .................. 71
10. Effect of Foot Shock on Subsequently Conditioned Avoidance Behavior .............. 82
11. Effect of Prior Foot Shock Stress on the Climbing Ability of Mice in the Avoidance Conditioning "Climbing Screen" .................. 83
12. Effects of Avoidance Conditioning on Stress-Induced Changes in Whole Brain 5-hydroxytryptamine and Norepinephrine Levels ............. 86
13. Mouse Strain Differences in Avoidance Conditioning .............................. 105
14. Failure of Five Different Stressors to Affect Locomotor Activity ....................... 117
CHAPTER I

OBJECTIVE

Since the denouement of the reticular versus synaptic theory of neural transmission in favor of Cahal's (1911) view that neurons form discrete units, and Loewi's (1926) and Dale's (1938) hypothesis of chemical neurotransmission, several substances have been proposed as neurotransmitter agents. In addition, these agents have been postulated to serve as modulators, in specific neural circuits, and, thereby to subserve distinct behavioral and physiological functions.

In order for a substance to be established as a neurotransmitter, certain criteria must be met. These can be summarized as follows:

1) The substance, as well as its precursors and metabolites, must be shown to exist in the central nervous system;

2) The substance must be released from nerve terminals by neuronal discharge or depolarization; and be metabolized by degrading enzymes or taken up by the nerve terminal after release;

3) The substance must produce a post-synaptic response, such as an inhibitory (hyperpolarizing) or excitatory (depolarizing) post-synaptic change in membrane potential.

A massive literature has accumulated with the objective of demonstrating that certain amines and amino acids meet these criteria, and, thus serve as neurotransmitter agents.
Included are 5-hydroxytryptamine (5-HT), dopamine (DA), norepinephrine (NE), epinephrine, acetylcholine (ACh), glutamine, aspartate, gamma-aminobutyric acid (GABA), glycine, and histamine (Agranoff, 1975). Recently, several peptides (amino acid chains), including substance P, methionine 5-enkephalin, neurotensin, and beta-endorphin, have been proposed as neurotransmitters or neuromodulators (Elde et al., 1976; Uhl et al., 1978).

A variety of methods have been developed and employed in the search for potential neurotransmitter agents. These can be summarized in three categories:

1) Anatomical: Enzyme fluorescence and immuno-histochemistry, and anterograde autoradiographic and retrograde horseradish peroxidase tracing, combined with a variety of lesion and biochemical methods.

2) Biochemical: Radio-enzymatic-and immuno-assay, chromatography, and the analysis of perfusates obtained from push-pull cannulae implanted in neural tissue; and,

3) Electrophysiological: Intra-and extra-cellular recording, chemical and electrical stimulation, and microiontophoresis.

By a combination of these methods it now seems certain that the monoamines, 5-HT, NE and DA, as well as GABA and ACh, serve as neurotransmitters in certain brain regions. Precursors and metabolites have been detected in brain, the monoamines are probably released by nerve terminals, and they are potent in affecting the membrane potentials of
presumed receptor cells. In addition, these substances are contained within neurons which are organized into discrete cell groups which have distinct axonal projection targets. Similarly, evidence is accumulating which indicates that glycine, aspartate, glutamate, and histamine, as well as some peptides also meet these criteria. Some of the above putative transmitter agents are anatomically organized into systems, that is, cell group clusters with distinct projection sites. These observations suggest that these systems also serve distinct roles in the regulation of specific physiological and/or psychological processes.

The overall objective of the present study is to determine the role of the noradrenergic, serotonergic, and cholinergic systems, as well as the putative gabaergic interneurons, in mediating an animal's response to stressful stimuli. These four neurotransmitters, along with DA, have been postulated as playing important roles in several physiological and behavioral processes (Goodwin and Sack, 1973). Thus, in the "reward centers" described by Olds et al. (1964), NE has been implicated in the "pleasure" experienced, and DA in the pleasure anticipated (Crow, 1972). The role of DA and NE systems in mediating positive reinforcement, however, is highly controversial (Fibiger, 1978). Both 5-HT (Crow, 1973) and ACh (Wise et al., 1973) systems have been implicated in negative reinforcement processes. Again, their specific roles remain a matter of intense debate (Lorens, 1978). In addition to their involvement in rein-
forcement processes, NE, DA, 5-HT, ACh and GABA systems have been hypothesized to play important roles in sleep (Jouvet, 1972), pain (Lorens, 1978), temperature regulation (Myers, 1974) and sex (Gessa and Tagliamonte, 1974).

The biochemical measurement of levels and aggression, (Karczmar, 1976 and Karczmar et al., 1978), of these substances in brain are relatively crude in the sense that they do not permit detailed descriptions of complex molecular changes and interactions. It is generally believed, nevertheless, that these substances are present in several compartments. They may be stored in vesicles, present in free-form in the synaptic cleft, or bound briefly to a post-synaptic cell membrane receptor where membrane changes in permeability are induced (Tranzer, 1973). Furthermore, some may be contained in blood, and in non-neural tissue, and may not be of neural origin. This is particularly true of 5-HT, 96% of which is located in enterochromaffin cells of the GI tract, platelets and mast cells.

At this level of analysis, a functional transmitter is that which has been released by neuronal discharges (or pharmacologically) and provides for information transfer post-synaptically by latering the receptor. In this regard, studies on transmitter levels give only a partial indication of functional activity and its link with behavior. However, there may be a distinct proportionality between the amount released and the amount stored. Although it is true that some regions of brain may be rich, and others poor, with regard to certain transmitters, some brain functions
clearly utilize many neurotransmitter systems (Karczmar, 1978). Therefore, a whole brain and brain part analysis of several neurotransmitters might be of value in determining their role in certain behavioral responses.

Techniques used to measure the turnover of neurotransmitter agents must meet rigorously controlled conditions of synthesis and degradation (Costa, 1971). Changes in the rate of synthesis and degradation of transmitters may occur along with changes in temperature and other physiological parameters, independent of transmitter release and functional significance (Neff et al., 1974). A fall in level may signify an increase in turnover rate, but turnover rate cannot be measured while levels are changing, since a change in level may mean that rate of synthesis does not equal the rate of degradation and that the steady state is not maintained, an essential condition for turnover rate determination. Since positive and negative feedback control of transmitter release has been documented, it is important to measure both concentration and turnover rates simultaneously.

In the present study, I have attempted to establish correlations between the brain concentrations and turnover rates of certain transmitter agents and specific behaviors emitted by mice. Earlier studies in our laboratory have demonstrated that brain levels of NE, ACh, and 5-HT fluctuate in an approximately direct relationship to each other across various genera of mice (Karczmar et al., 1973). The goals
of the present study, thus, were fivefold: 1) to subject different strains of *Mus musculus* to a variety of stressors including foot shock, glossopharyngealctomy, olfactorybulbectomy, enucleation, and isolation; 2) to study the effects of these stressors on brain NE, 5-HT, ACh, and GABA concentrations and turnover rate; 3) to study the effects of stress on conditioned avoidance learning; and, 4) evolve an hypothesis relative to the function of central neurotransmitters in the stress and coping syndrome; 5) to study the inate differences in neurochemistry and behavior found among various strains of Mus.
CHAPTER II

METHODS AND MATERIALS

A. ANIMALS

All experiments used mice of one of two strains: *Mus musculus* CF-1 and *Mus musculus* C57BL/Bailey (BY). The CF-1 mice were obtained from Carworth Farms Laboratory (Porthage, Mi.), while the C57BL/BY mice were obtained from the Jackson Laboratory (Bar Harbor, Maine). Only male mice weighing 25-35 grams (g) were used. The animals were housed 10 per cage in an illumination (12 hour dark-light cycle; lights on at 0700 hours) and temperature (75± 5 degrees F) controlled room. Food and water were available ad libitum in the home cage.

B. BEHAVIORAL MEASURES

1. Avoidance Conditioning "Climbing Screen"

The avoidance conditioning climbing screen was constructed of Plexiglas and consisted of 5 pairs of base chambers and inclined runways (A 1-5 and D 1-6), respectively (Fig. 1 and 2). The chambers and runways were arranged sequentially in a stairway fashion, the chambers communicating with the runways by means of solenoid-operated gates (Fig. 2, J). Each runway, inclined at an angle of 35 degrees, was 12 inches (in) long, 3 in. wide and 3 in. high. The dimensions of each
Figure 1. Photograph of the automated avoidance conditioning "climbing screen" apparatus.
Figure 2. Diagram of automated avoidance conditioning "climbing screen". A 1-5: base chambers; D 1-5: climbing ramps; J: guillotine doors, or gates; B 1-5: elapsed timers for base chambers; C 1-5: elapsed timers for climbing ramps; E and F: reset buttons; G, H and I: milliamperemeters for control of shock intensity.
base chamber were 3 in. by 2 in. by 3 in. high. Grids made of wire (3.0mm in diameter), fastened 1/8 in. apart, constituted the floors of the base chambers and runways. The latter were composed of four sections each. The grids could be electrified successively. The programming controls consisted of delay timers (Fig. 2, G and H) which operated the closing and opening of the gates. They were used to set the time interval between the opening of a gate, and the activation of the base chamber grid (Fig. 2, G and H). The stimulus parameters, and the distributor for randomizing the shocking current to the grid floor, were controlled manually by the experimenter. Light beams which impinged on photocells installed at the doorways to and from each base chamber served to provide a record of the time spent by the mouse in each of the base chambers, as well as the rate at which the mouse climbed from one chamber to the next. The beams activated ten elapsed-time meters (Fig. 2, B and C: calibrated in 1/10 second). The stimulus parameter was 1.3 milliamperes.

Each conditioning trial was run as follows. The mouse was placed in the lowest chamber, and its exit gate, as well as that of the next chamber, was opened 60 seconds (sec.) later. The chamber grid was electrified after an additional 5 sec. had elapsed. The electric shock then was applied at 10 sec. intervals to the four consecutive sections of the runway grid. Immediately after the animal entered the next base chamber, the entrance gate closed behind it and the entire cycle was repeated. In the course of one completed
trial the mouse proceeded through a linear series of five base chambers alternated with five ramps.

During each trial the elapsed-time meters recorded the length of time the mouse spent in each base chamber after the opening of the gate. These elapsed times are referred to as "base" times. Thus, a "base" time of more than 5 sec. indicated that the mouse received shock to its feet prior to leaving (escaping) the chamber; times shorter than 5 sec. indicated an avoidance response occurred. The time meters also recorded the length of time that a mouse spent on the ramp; these elapsed times are referred to as "climbing" times. Each trial with one mouse, therefore, yielded ten readings consisting of five "base" and five "climb" times.

These readings could be manipulated and presented in various ways. The "climbing" and "base" times could be plotted individually for each mouse, as it progressed from level to level, and from trial to trial, each plot constituting a 50-point graph. Various methods of analysing the data also could be employed. The "base" and "climb" times for ten trials could be averaged per mouse, or for the ten mice used per experimental group. In the latter case, an average value based on five hundred readings (ten trials per mouse; five "base" and five climb times per trial) could be averaged per level for the ten trials, either for the individual mice or for the the ten mice in each experiment. The values calculated by these methods did not reflect changes occurring from trial to trial. Accordingly, the "base" and "climb" times could be averaged for each ten mice. Thus, for
each successive level and trial, or five "base" and "climb" times, could be averaged for each group of ten mice, five values each per trial, for each of the ten trials, or the number of avoidance responses (CAR's) could be averaged for each mouse or group of mice for each trial. Any reading less than 5 sec. indicated avoidance, (Scudder et al., 1969).

2. Locomotor Activity "Photoactometers"

Activity was recorded in a constant environmental chamber (75 ± 5° F) by means of Lehigh Valley Electronic Photoactometers (Fogelsville, Pa.) measuring 18 in. in diameter and 12 in. high with a wire mesh floor. Photocells (n=6) were situated 1 in. from the floor, and 2 in. apart around the cylindrical chamber. The chambers were dark with no food or water available. One animal was placed in the chamber and left there for 1 hour. Each beam interruption was recorded by digital counter. Activity was recorded every 10 min. for the first hour. The data were reported as activity counts per 10 min. period and as the total activity counts for the 60 min. session. Typically, the activity level would decrease over time.

3. Agression (Isolation Induced)

A "round robin" (Valzelli et al., 1968) technique was
used to measure the effects of isolation on aggression. The mice were housed individually for a minimum of two weeks in Plexiglas cages in constant temperature (75 ± 5°F). A 12 hr. light-dark cycle was maintained, and food and water was available ad libitum. The mice were not handled during the isolation period. Following the isolation period, ten mice were exposed, one against the other, until each mouse had been exposed to every other mouse. Thus, a total of forty-five encounters constituted one series of experiments. The "fighting" cages were constructed from stainless steel and measured 5 in. by 7 in. x 5 in. high. Sawdust was placed in the bottom of the cage. Two mice were placed in the cage separated by a removable metal divider. The "fighting" cages were washed and fresh sawdust provided between each behavioral observation period. Two of these mice were placed in the fighting chamber with a steel wall between them. After 15 sec, the wall was pulled out and the two mice allowed to fight. The occurrence of a "fight", the aggressive mouse, and the latency in sec. from the time of removal of the separation wall to the first appearance either fierce wrestling or biting was recorded. After the experimental procedure was completed, the two mice were returned to their individual cages, the fighting chamber was cleaned, and two different mice were allowed to fight. In this way, a "round-robin" of fights among the ten mice was performed. After each mouse had fought every other mouse (a total of 45 possible fights), the animals were sacrificed.

Two measurements were recorded during each episode; the
latency in seconds for the animals to attack, and the percent of animals from each group of ten who fought. The total number of "fights" observed also was recorded. In this manner, very aggressive or very timid mice can be discerned. The encounters were numerically arranged by the investigator to make certain that no animal fought twice in a row. Thus, the learning variable to fight was somewhat controlled, although the latency to fight was decreased as the experiment progressed through the forty-five encounters.

4. Foot Shock

The mice were given shocks of 0.05 millivolts for 2 sec. in Lehigh Valley Electronics Model 1002 shock chambers. The chambers were made of Plexiglas with a wire grid floor. Each rod was separated by 5 mm and comprised the entire cage floor. The animals were not able to escape the shock. Sixty shocks were administered per experiment. There were twelve animals per experiment. The shocks were administered manually by the investigator and several behavioral indices were noted, including catatonia, biting the cage, tail rattling, and piloerection. Catatonia was defined as a rigid, fixed position with no movement or tremors. At times animals receiving shocks would not change their position from one shock to the next. As the experiment progressed most animals became more and more "catatonic". For most of the experiments described below the animals were taken directly from the shock cages and placed in another testing apparatus
(activity wheel or the "climbing screen") or sacrificed for biochemical analysis, as detailed below.

C. BRAIN EXTRACTION AND DISSECTION

Each mouse was weighed, microwaved as described below, and rapidly decapitated using a pair of sharp scissors. Its brain was immediately extracted in the following manner.

A midline incision was made through the skin covering the cranium, thereby revealing the entire dorsal surface of the skull. Inserting the tips of a pair of fine scissors into the opening at the caudal end of the cranium, a dorso-lateral cut was made, proceeding rostrally first through the cartilage bordering the right side of the brain, then through the left side, both cuts meeting directly over the olfactory bulb region. The cranium then was removed exposing the entire dorsal aspect of the brain. The brain, including the olfactory bulbs, was gently lifted out of the skull with a spatula. In the process of removing the brain, the spatula was used to sever the brain from the cranial nerves, and the hypophysis, which were the only elements holding the brain in place within the skull. At this point the brain either was immersed directly into a flask containing liquid nitrogen or divided into parts, depending on whether the whole brain or parts of brain were to be used. When whole brains were used, the entire process of excision and immersion into liquid nitrogen took less than one minute. In those experiments requiring brain parts, upon removal of the whole brain from the skull, the following dissection was rapidly performed.
The caudal ends of the neo-cortex were lifted up and forward exposing the axonal connections between the diencephalon and the telencephalon, namely, the striae terminalis and the internal capsulae. Severing the brain at these points on both the right and left sides separated the telencephalon (T) from the rest of the brain. The telencephalon, thus obtained, included neo-cortex, the olfactory bulbs, the hippocampus, and the basal ganglia (striatum and amygdala). The midbrain-diencephalon (M-D), including the thalamus, hypothalamus and midbrain, was obtained by sectioning the brain at a point immediately rostral to the cerebellum and caudal to the inferior colliculi. Thereafter, the cerebellum was detached from the M-D and combined with the remaining brain tissue, the pons-medulla, which terminated caudally at a point immediately rostral to the first vertebra. As each of these parts were isolated, they were immediately immersed in liquid nitrogen. The process of sacrifice, brain extraction, division into parts, and immersion in liquid nitrogen took less than two minutes. Due to the small amounts of tissue obtained, pooling of some brain parts was necessary for biochemical analysis. In general, studies employing brain parts required parts from 2-4 different mice.

Microwave irradiation and immersion into liquid nitrogen was found to be the best way to sacrifice and minimize metabolic activity (Richardson and Scudder, 1976).
D. TEMPERATURE MEASUREMENTS

Both core brain temperature and body temperature were measured throughout these experiments. Rectal temperature was measured with a Yellow Springs Instrument (Antioch, Ohio) Model 4 3TC telethermometer with a 0.25mm diameter thermistor probe (#40). The probe was inserted rectally (1 cm) and the temperature recorded before, during and after various behavioral and biochemical manipulations.

Immediately following irradiation, the mice were decapitated and the thermistor probe was inserted 1.5 cm into the core of the brain through the foreman magnum.

E. SURGICAL PROCEDURES

1. Bilateral Glossopharyngeallectomy

A bilateral glossopharyngeallectomy was performed as follows. The mouse was anesthetized with Nembutal Sodium, 60 mg/kg, intraperitoneally (i.p.). The animal was shaved and tied on its back on an operating table. When the animal was sufficiently anesthetized, a 5/8 in. transverse incision was made in the throat with sterilized instruments. The submaxillary gland was lifted with a dull probe and held by a hemostat. A dull probe and sharp forceps were used in teasing muscles and fascia until the trachea was visualized. The trachea then was lifted slightly laterally and, at the site of the carotid sinus, between the junction of the internal and external carotid arteries, the glossopharyngeal nerve identified. An A.C. Baker (London) dissecting
microscope was used to locate the nerve. The glossopharyngeal nerve was lifted by means of a microhook and was then cut with a microscissors. A 9.3 mm section was excised from the nerve between the carotid and lingual branch. An identical procedure was carried out on another group of mice with the exception that the glossopharyngeal nerve was merely identified and probed but not cut. Two 7 mm wound clips were used to close the incision. The mouse then was placed on its stomach in a clean cage. It was warmed post-operatively by a 100 watt lamp until it recovered from the anesthetic. After 2 hours the animal was replaced in its home cage.

2. Enucleation

CF-1 mice were enucleated 3 days after birth as follows. An incision was made around the entire orbital area and the eye balls removed from their sockets with a microscapel. Very little bleeding occurred and, thus, a needle and fine nylon thread were used to close the incision without packing the wound. The animals were placed with their mothers immediately after the operation until they were 20 days old. At this time they were separated and housed in cages. An incision around the orbital area was made on the sham operated animals, the eye balls were not removed, but the incision was closed as above.

3. Olfactory Bulbectomy

A bilateral olfactory bulbectomy was performed as
follows.

CF-1 mice were anesthetized with Nembutal Sodium (60 mg/kg, i.p.). Pontocaine (5 mg/kg) was administrated subcutaneously and an incision made medially from the level of the auricles to 1/8 in. from the tip of the nose. The skin was pulled to one side, exposing the skull. Two 1/8 in. holes were drilled bilaterally in the skull, exposing the olfactory bulbs. The durameter was incised, and the bulbs removed by means of suction through a micropipette. The hole in the cranium then sealed with bone wax to prevent bleeding and infection. The wound was closed with a 7 mm wound clip. The animals then were placed in a clean cage and warmed with a 100 watt lamp until they recovered from the anesthetic. An identical (Control) operation was performed on another group of mice except that the olfactory bulbs were not removed.

F. BIOCHEMICAL DETERMINATIONS

1. Norepinephrine (NE) and Serotonin (5-HT) Concentrations

The fluorometric technique of Maickel et al. (1968) was used to measure the levels of NE and 5-HT. The animals were sacrificed by microwave irradiation utilizing a Litton Industries (Chicago) Model 350 microwave oven. The mice were placed in the oven for 5 sec. of whole body irradiation. They then were decapitated and their brains rapidly removed and placed in liquid nitrogen. In experiments involving brain part, the brain was dissected and studied.
as described above (section C). The sample then was weighed and homogenized in a volume of acidified N-butanol equal to the weight of the sample. A Talboys Instrument Corp. (Emerson, N.J.) Model 103 motor set at speed 60, and a spinning Teflon pestle in a size A Thomas (Phila., Pa.) grinding tube, was used to homogenize each brain sample for 1 min. The homogenate then was transferred to a 13 ml test tube and shaken (Eberbach, Ann Arbor, Mi.) automatically for 10 min. The homogenate was then centrifuged in an International Equipment Co. (IEC; Needham Hts., Mass.) centrifuge at 2000 RPM for 20 min. The supernatant then was transferred to test tubes containing 7 ml heptane and 0.2 ml 0.1N hydrochloric acid (HCl). This mixture was shaken for 10 min, as above, then centrifuged for 20 min. The organic phase then was discarded, and the acid phase containing NE and 5-HT was prepared for fluorometric analysis. External and internal standards and blanks were carried through each experiment to determine the amount of NE and 5-HT lost, and the amount of contamination picked up during the procedure. One microgram (µg) per ml of NE and 5-HT was added to the recovery tubes, while the recovery blanks contained 0.1 N HCl in equal volume to establish standard curves.

Following the above, 0.1 ml iodine was added to the acid extract. Exactly 2 min later 0.2 ml sodium thiosulfite (Na₂SO₃) was added. Acetic acid (10N) in the amount of 0.2 ml was added, and the acid extract heated in boiling water (H₂O) for 2 min. The samples then were transferred into 10 mm optical cuvettes and measured using an Aminco Bowman
Spectrophotofluorometer (Silver Springs, Md.). Fluorescence was read directly from a photomultiplier photometer (Aminco Bowman) at an activation wavelength of 385 mm and an emission wavelength of 485 mm.

The procedure used to determine the level of 5-HT was identical to that for NE up to the preparation of the acid extract. Para-opthalaldehyde (0.6 ml) was added to each acid extract sample, followed by heating in a boiling bath for 10 min. The samples then were transferred to 10 ml cuvettes and placed in an Aminco Bowman spectrophotofluorometer. Fluorescence was read at an activation wavelength of 360 mm and emission wavelength of 470 mm. The following formula was used to calculate the amount of NE and 5-HT found in brain, expressed as µg/g wet weight.

\[
\frac{X - Y}{Z - Y} \times \frac{1000 \mu g}{W} = \text{Amine Concentration (µg/gm)}
\]

where

- X = fluorescence reading of sample
- Y = fluorescence reading of recovery blank
- Z = fluorescence reading of recovery (non-tissue)
- W = weight of tissue sample in mg.

Recoveries were calculated by the formula.

\[
\frac{Z - Y}{A - B} \times \frac{2.8 = \text{ml butanol}}{2.5 = \text{ml butanol}} \times \frac{0.2 \text{ HCl}}{0.1 \text{ HCl}} \times 100\%
\]

where:
- B = fluorescence reading of standard blank
- Y = fluorescence reading of recovery blank
- Z = fluorescence reading of recovery
- A = fluorescence reading of standard
Recovery of 65-75% was realized using this technique. The sensitivity of this technique is such that a minimum of 4.0 ng/g wet weight NE, and 5.0 ng/g wet weight 5-HT could be detected reliably.

2. Gamma-Amino-Butyric-Acid (GABA) Level

It was determined in our laboratory (Richardson and Scudder, 1976) that 10 sec whole body exposure to microwave irradiation (600 watts; \(2.45 \times 10^9\) Hz) will completely stabilize for more than 15 min the post-mortem levels of GABA in mice weighting up to 36 g. After sacrifice the body was placed under tap water, and the brain, or dissected parts, placed into liquid nitrogen. Baxters (1973) method for determining GABA level was used in these experiments.

A Talboys Instrument Corp. Model 103 motor (set at speed 60), with a spinning Teflon pestle in a size A Thomas grinding tube, was used to homogenize each brain, or brain part, for 1 min in ice-cold 75% v/v ethanol slightly acidified with several drops of 5 or 6 N HCl per 400 ml. Five ml. of ethanol solution was used for whole brain (2.5 ml, for brain parts). The homogenate was centrifuged for 1 hr. at 19,000 RPM in an IEC Model R20 centrifuge refrigerated to \(-10^\circ C\). Evaporation of the ethanol was enhanced further by a manifold directing filtered air into the vials via an air pump and 1 cm diameter rubber tubing. The evaporation process was continued overnight to insure that no trace of ethanol was left. The residue then was taken up in 1 ml of 0.1 M
potassium pyrophosphate (KPP04) buffer, prepared by placing 38.45 of KPP04 in 900 ml distilled H20, adjusting the pH to 8.6 with 5 or 6 N HCl and filling to 100 ml with H20. The buffer residue mixture was shaken for 1 min in a Scientific Products Deluxe Mixer (Model 07316), set at speed 4, then centrifuged as described above (except at 5°C). For whole brain analysis 0.1 ml of the supernatant was transferred to a test tube containing 2.5 ml of KPP04 buffer. For brain part analysis, 0.5 ml of the supernatant was placed into 2.1 ml buffer. Subsequently 0.15 ml of 0.004 M nicotinamide-adenine dinucleotide phosphate (NADP) sodium salt was added to each test tube. NADP solution was prepared by mixing 33.33 mg NADP per 10 ml distilled H20 and neutralizing to pH 7.9 with reagent grade solid sodium bicarbonate (NaHCO3). After adding 0.1 ml GABA-ase (Sigma Chemical Co., St. Louis; G2006) preparation to each tube, and mixing briefly on the mixer, the solutions, including the blank and reference standards (discussed below) were each poured into 10 mm optical pathway cuvettes. The optical density of each sample was measured at 340 mm wavelength using a Beckman model DU spectrophotometer calibrated to indicate zero absorbance with a cuvette filled with distilled H20.

The reaction by which GABA was measured then was initiated by the addition into the cuvette of 0.15 ml of 0.02 M alpha-ketoglutarate (AKG) (Sigma K-1875) which was prepared by mixing 23 mg. of AKG into 10 ml of KPP04 buffer. The pH was 7.9 or slightly higher without adjustment. After the AKG was added, the optical density increased rapidly and
linearly for about 8-9 min which varied less than 2% for 3-4 min before starting to decline rapidly. There was slight \( \Delta OD_{0.004} \) increase in optical density not attributable to the reduction of NADP in the reaction utilizing GABA. Because the initial reading of absorbance must be made before the reaction is started by adding AKG, there was an even larger increase in optical density (approximately \( \Delta OD_{0.016} \)) resulting merely from the addition of the AKG. To account for these two effects, each run contained two blanks consisting of reaction mixtures prepared as for whole brain analysis, except with 0.1 ml buffer substituted for the GABA solution. The average value of blanks was added to each initial absorbance value of all samples and standards. The resulting sum for each sample and standard then was subtracted from its corresponding final absorbance reading to yield the quantity "change in optical density" used in the following formulae.

Reference standards of known quantities of GABA were included in each run to provide a means of calculating the quantities of GABA in test samples. The amount of GABA in the tissue sample \( (X) \) is related to known amount of GABA in the standard \( (K) \) in the same proportion as the change in optical density of the sample \( \Delta OD_X \) which is related to the change in optical density of the standard \( \Delta OD_K \).

Thus:

\[
\frac{X}{K} = \frac{\Delta OD_X}{\Delta OD_K}
\]
The quantity $X$ is in units of mg/ml because the total GABA content of the sample is taken up in 1 ml of KPP04 buffer.

In order to report GABA content in terms of mg/g tissue, wet weight, both sides of the equation, are divided by the tissue weight ($W$).

\[
\frac{X}{W} = \frac{K}{W} = \frac{\Delta OD_x}{\Delta OD_K} = \frac{\Delta OD_x}{W} = \frac{K}{\Delta OD_K}
\]

Recovery of 85-90% was realized using this technique.

This is the most reliable and sensitive method available.

3. Acetylcholine (ACh) Level

A modified technique of Jenden et al. (1972) was used to determine the endogenous levels of ACh in the brain (Haubrich and Reid, 1974). The assay is based on the conversion of choline to $^{32}$P labelled phosphorylcholine in the presence of excess ATP-$^{32}$P. This reaction is catalyzed by the enzyme choline kinase. The phosphorylcholine-$^{32}$P was measured by ion exchange chromatography. Choline and ACh are isolated initially from brain by high-voltage paper electrophoresis.

The animals were sacrificed in a microwave oven, as described above, and their brains rapidly removed and frozen in liquid nitrogen. The tissue subsequently was homogenized, as
described above, in 2.5 ml of 15% formic acid and incubated on ice for 20 min. after shaking for 10 min. The homogenates then were centrifuged in polypropylene centrifuge tubes (Omega) at 0-5°C for 15 min at 7000 RPM using an IEC refrigerated centrifuge. The supernatant then was decanted into a 50 ml glass stoppered centrifuge tube and stored on ice. The pellet then was resuspended in 10% formic acid (2 ml for each gram of tissue) incubated for 20 min on ice and recentrifuged as above. The supernatant fluids were combined with an equal amount of ether which was saturated with water for 5 min. After the phases were separated with a separatory funnel the organic layer was aspirated and discarded. The organic solvents which remained were evaporated in the formic acid phase by placing the chilled samples under a stream of air. The samples then were centrifuged for 10 min at 2000 RPM and 0.2 ml of the supernatant fluid was transferred to a 13 ml centrifuge tube. The samples then were freeze dried (Labco Freeze Dryer) for 24 hr. Following freeze drying, 50 μl of double distilled H₂O was added to the samples. The mixture then was vigorously stirred with a glass rod and centrifuged at 2000 RPM for 5 min. Ten μl of the clear supernatant fluid was spotted on a line drawn on a sheet of Whatman (Scientific Products) 3 mm chromatography paper. The line was 10-15 cm from the end of the paper and perpendicular to the direction of current flow. The spots were placed approximately 2 cm apart. For later identification of the unknown samples, several marker spots containing 10 μl of the tissue extract plus 5
μl of the choline and Ach marker were placed intermittently between the sample spots. Internal standards were prepared by spotting. Dilute choline (10 μl) and Ach (10 μl) standards were placed on the electrophoresis paper and handled in parallel with the tissue samples. The chromatography paper was placed on a flat plate electrophoresis unit with its origin toward the positive electrode. The paper then was moistened and the electrophoresis set to run at 500 volts for 3 hr.

After electrophoresis, the paper was dried in air and the marker spots identified by placing the paper in a closed cylindrical glass tank (2 ft dia, 2 ft high) containing 25 g of crystalline iodine. When the marker spots came into contact with the iodine vapor, a brown color appeared within 1 min. These spots were marked with a pencil. Using the standards as a guide, the positions of choline and Ach were identified. The spots containing tissue choline and Ach then were cut out from the paper. The papers thus obtained subsequently were placed in culture tubes (12 x 75 cm) containing 10 ml distilled water. The amines were eluted by shaking for 5 sec with a Vortex mixer. The samples then were incubated for 15 min and a 0.5 ml aliquot of the eluate was transferred to another culture tube containing 0.10 μl of concentrated ammonium hydroxide. The tubes then were mixed and heated in boiling H₂O for 20 min to hydrolyze the Ach. The samples then were evaporated to dryness by heating under vacuum at 50-60°C. At this point an enzyme-substrate
solution was prepared. The reagents consisted of 4 parts ATP, 5 parts sodium chloride (NaCl) solution and 1 part choline kinase. This mixture was pre-incubated for 30 min at 37°C to phosphorylate any residual choline present in the enzyme. After preincubation, 0.1 ml ATP-32P was added. The solution then was centrifuged for 5 min at 2000 RPM. Following the centrifugation, 0.1 ml of the enzyme-substrate solution was added to the tubes in which the eluate from the electrophoresis paper was evaporated. The solution was mixed to dissolve the choline. The samples then were incubated at 37°C for 3 hr to achieve quantitative conversion of choline to phosphorylcholine. After incubation 10 µl of phosphorylcholine and 1.5 ml of Tris (Sigma) buffer containing magnesium sulfate (MgSO₄) was added to each tube. The contents of the tubes were added to a Dowex 1 column and washed with an additional 4.5 ml of Tris buffer. The eluate was collected in a counting vial and measured in a Beckman liquid scintillation counter (Model 17265). The reagent blank was prepared by eluting a blank section of the electrophoresis paper and processing the eluate along with the tissue samples. The external blank contained only the enzyme. The substrate mixture was added to test tubes treated in manner identical to that for the preparation of the standards. The recovery was calculated using the following formula:

Percent Recovery = \[ \frac{\text{CPM}_{\text{IS}} - \text{CPM}_{\text{ES}}}{\text{CPM}_{\text{IS}} - \text{CPM}_{\text{RB}}} \times 100 \times 2 \]
where $CPM_{IS} = cpm$ Internal Standard

$CPM_{ES} = cpm$ External Standard

$CPM_{RB} = cpm$ Reagent Blank

$CPM_{EB} = cpm$ External Blank

A recovery of 70-80% was realized utilizing this technique.

The concentration of choline or acetylcholine was calculated using the following formula:

\[
\text{nmoles/g tissue} = \frac{(1 \text{ nM}) (CPM-CPM_{RB})}{CPM - CPM_{RB}} \times \frac{F}{\text{grams of tissue}}
\]

where $CPM_F = cpm$ tissue sample

4. 5-hydroxyindole acetic acid (5-HIAA) level

The brains or brain parts were homogenized as described above for the determination of 5-HT and NE. The procedure used for the determination of 5-HIAA was identical with that of 5-HT up to the aspiration of the organic phase containing heptane and butanol. Instead of aspirating off the organic phase, 8.5 ml of the heptane phase was transferred to a 13 ml tube. To these tubes 0.5 ml of 0.033M NaHCO$_3$ was added. The mixture was shaken for 1 min as described above. The tubes were then centrifuged for 5 min at 2000 RPM. The organic phase was aspirated and discarded. The aqueous
phase was mixed by vortexing. The mixture then was heated in a H₂O bath at 100°C for 10 min. The 5-HIAA levels then were determined as above in an Aminco-Bowman Spectro-photofluorometer at 360 millimicrons activation wavelength and 470 millimicron emission wavelength. Standard and recovery tubes were carried through the experiment as with one 5-HT determination. A recovery of 75-80% was realized using this technique.

5. ACh Turnover

Estimation of the ACh turnover requires the simultaneous determination of endogenous and tracer amounts of both ACh and choline (Haubrich and Reid, 1974). A trace amount of tritiated hydrogen (³H) labelled choline was injected intravenously into the tail vein of the mice. Brain ACh turnover was estimated from the proportion of ³H-choline converted to ³H-ACh in the brain at various times following injection. The total concentration of choline and ACh in brain did not change significantly following injection of ³H-choline. The initial rate at which the concentration of ³H-ACh rises represents an estimate of the rate of synthesis of ACh. The entire time course (15 min) was utilized to estimate the turnover rate using the following method. The rates of synthesis and degradation of acetylcholine equal V₁ and V₂ nmole/g/min respectively. If x and Y denote the total concentrations of choline and ACh and an asterisk denotes the labelled variant, then:
\[
\frac{d x}{d t} = V_1 \frac{x^*}{1x} - V_2 \frac{y^*}{-1y}
\]

The assumption that there are uniform pools of choline and ACh is implied in this equation. At steady state, \( V_1 = V_{-1} = V_1 \), or the turnover rate.

Hence

\[ \frac{d y^*}{d t} = V \frac{x^*}{x} - \frac{y^*}{y} \]

Measurements were made of \( s, x^*, y \) and \( y^* \) at each time point. Sacrifice times were 30 sec, 1 min, 2 min, 6 min, post-injection. The choline and acetylcholine concentrations were determined utilizing the same extraction procedures described above for the endogenous levels of choline and acetylcholine except that no 32p phosphorylcholine was added to the tubes. The paper spots containing \(^3\)H-choline and \(^3\)H-Ach were placed in culture tubes and 2.0 ml distilled H\(_2\)O was added to elute the amines. The mixture was then placed in 10 ml of "Cocktail D" which contained 5 mg PPO, 100 mg Naphthaline in 100 ml of Dioxane. The amount of radioactivity in the sample was measured in a Beckman Liquid (LS 250) Scintillation counter and converted to counts per minute (CPM) by multiplying the CPM times a correction factor of 1.333 and multiplying by the efficiency of the machine (which was 85\%). The conversion factor gives a reading in nmoles/gm/min. A straight line is obtained when \( \frac{d y^*}{d t} \) was plotted against \( \frac{x^*}{x} - \frac{y^*}{y} \).
6. Norepinephrine Turnover

Radioactive $^3$H-tyrosine was dissolved in 50% ethanol. The solution was freeze dried and the residue was dissolved in saline so that an injection of 1 ml represented 0.8 mc/kg of L-tyrosine. At selected times after the injection, mice were decapitated and the blood was collected in beakers containing heparin. The brains were removed as described above. The specific activity of tyrosine and NE were determined by a modified procedure described by Costa (1971). The plasma or brain samples were homogenized as above in volumes of 0.4N perchloric acid containing 0.05 g sodium metabisulfite. After centrifuging at 200 RPM for 10 min, 4.7 ml of supernatant was added to 0.3 ml of 10 M potassium acetate. After a second centrifugation, 4 ml of the clear supernatant were placed on a Dowex 50 - X4 200 to 400 mesh column (25 mm x 26 mm$^2$). Tyrosine is found in the sample effluent and in the effluent after the addition of 4 ml of 0.1 M sodium acetate buffer pH 4.5 containing 0.1% disodium edetate. The combined effluents were designated as Fraction A. NE was eluted using 15 ml of 0.4 N HCl and designated fraction B. Fraction A, containing tyrosine, was brought to pH 8.3 by adding 0.75 ml of 3 M tromethamine. Alumina (Woelm, neutral, grade 1), about 500 mg, washed with 5 ml of 0.1 M disodium edetate prior to use was added to the above solution. The samples were shaken for 10 minutes as above and then centrifuged at 2000 RPM as above. The supernatant was titrated to pH 1.5 and 7 ml were placed on a Dowex 50 X4 200-400 mesh
column. Tyrosine was eluted by adding 7 ml of 0.1 M tri-

basic sodium phosphate. Stable tyrosine was assayed by

forming a fluorophor with 1-nitro-2 napthol. An aliquot of

the final solution was added to a Triton X-100 solution

containing 1 liter Triton X-100, 2 liters toluene, and 23

g 2, 5-diphenyloxozol and the mixture was counted in a

Beckman (LS 250) liquid scintillation counter.

The general equation used for measuring the change of

specific activity of the monoamines over time is as follows:

\[
\frac{dM}{dt} = K_m (AA-M)
\]

where M is the specific activity of monoamine, AA the speci-

fic activity of the amino acid and \(K_m\) the rate constant of

the monoamine pool. To estimate the synthesis rate of the

monoamines, the amino acid and monoamines were measured at

1, 2, 4 and 8 hr after and tailvein injection. Then the

average AA and M values were plotted on semi-logarithmic

graph paper and a best fit line was drawn connecting the

points. The graph was divided into consecutive 20-minute

intervals and \(K_m\) calculated for each interval. The synthe-

sis rate of NE was determined by multiplying \(K_m\) by the

steady-state concentration of the amine.

G. STATISTICAL ANALYSIS

A t-test (two-tail) for independent means (Hill, 1961)

was used to determine significant differences between con-

tral and experimental groups with regard to neurotransmitter

correlation and turnover, locomotor activity and number of
fights in the aggression test (Chapter V). The same t-test but one-tailed, was used to analyze the date in the drug study (Chapter IV). A Chi-square (Senter, 1969) was employed in the aggression study (Chapter I) with regard to the latency (sec) to fight. A two way analysis of variance (ANOVA) (Winer, 1962) was used to analyze the avoidance data (Chapters III-VII). A regression analysis was performed on the data with regard to the synthesis rate of ACh and NE to determine validity of the best fit line.
CHAPTER III

CHANGES IN CENTRAL TRANSMITTER LEVELS AND
TURNOVER ASSOCIATED WITH STRESS

A. INTRODUCTION

A considerable amount of research has centered on the physiological and psychological effects of stress. One of the problems surrounding this area of research in mammals derives from the lack of a generally accepted definition of the stress response. At the present time it is not possible to define stress in terms of a single response or set of responses; nor is there a common set of variables which change consistently as a result of subjecting an individual to stressful stimuli. On the other hand similar autonomic and endocrine responses, for example, increased heart rate, increased blood pressure, and elevated levels of circulating corticosteroids, are seen following some stressors, such as exercise, fighting and pain. These three responses, thus, have been considered as indicators of "stress".

The studies by Cannon (1935) and Selye (1935), which describe in detail certain physiological changes which occur in response to noxious stimuli and tissue damage, opened the door to studies of the way in which an organism "copes" with a particular stressor. Adaptation and maintenance of homeostasis by an organism is the basis for much of this work, particularly that concerned with the effects of stress on
neurotransmitter metabolism and steroid interaction. The objective of these latter studies was to elucidate the role of distinct central systems in mediating physiological and behavioral ("coping") responses to stress.

Studies of the effects of stress induced by different conditions or stimuli on norepinephrine (NE) and 5-hydroxytryptamine (5-HT) levels and turnover in the central nervous system have produced inconsistent results. An increase in brain 5-HT following different types of stress has been observed by several investigators. For example, an increase in brain 5-HT level was found by Ladisich (1974, 1975) and Curzon (1974) after foot shock stress in rats. Immobilization stress also has been reported to increase brain 5-HT concentrations (Heide and Peters, 1970). Morgan et al. (1972), furthermore, reported a small but significant increase in brain 5-HT after food deprivation. Cold and heat stress also has been reported by some authors to increase 5-HT brain level (Dixit and Buckley, 1969; Menon and Dandiga, 1969; Francesconi and Mager, 1974).

Other workers, however, have reported exactly opposite results following the exposure of animals to stress. For example, Salama and Goldberg (1969) found a reduction in brain 5-HT after foot shock stress. A decrease in midbrain 5-HT turnover was reported by Brown et al. (1974) after hypoxia stress. Curzon (1971, 1972) reported a decrease in whole brain 5-HT level after immobilization stress, while he (1971) also reported a decrease in 5-HT whole brain concentration after both heat and cold exposure. Therefore, Curzon found an increase
or decrease in brain 5-HT depending on the nature of the stressful stimuli. Exercise was shown by Anakhina (1973) to decrease brain level of 5-HT. Radulovacki (1976) demonstrated that paradoxical sleep deprivation resulted in a decrease in brain 5-HT and cerebrospinal fluid (CSF) 5-hydroxyindole acetic acid (5-HIAA) concentrations.

As might be expected from the above discussion 5-HT turnover rate has been found by many investigators to increase during and following stress. For example, several workers (Bliss et al., 1968; Modigh, 1974; Curzon, 1974) have reported increases in 5-HT turnover after foot shock stress. Curzon (1974) and Bliss et al. (1972) showed that immobilization stress increased 5-HT turnover in rat brain. Stress associated with fighting and isolation has been reported to increase (Welch and Welch, 1970) and decrease (Eleftheriou and Church, 1968) 5-HT turnover, respectively. Modigh (1974), Radulovacki (1973) and Morgan et al. (1976) reported that rapid eye movement (R.E.M.) sleep deprivation caused an increase in 5-HT turnover. Crowding was reported by Bliss and Ailion (1969) to result in an increase in 5-HT turnover. In contrast, isolation induced stress in their experience did not affect 5-HT turnover.

Some investigators, on the other hand, have reported that stress does not affect brain 5-HT level or turnover. For example, Bliss et al. (1968, 1972) and Baldessarini (1972) found no change in 5-HT level after foot shock stress. Curzon (1973) found no change after immobilization stress.
Exercise was shown to have no effect on 5-HT levels (Salama and Goldberg, 1969; Goldberg and Salama, 1970). Stern et al. (1971) found no change in 5-HT level after prolonged periods of sleep deprivation. An unchanged 5-HT turnover was reported after immobilization and exercise by Corrodi et al. (1971) and Goldberg and Salama (1970), respectively.

The literature concerning changes in brain NE metabolism following stress also is extensive but inconclusive. A number of investigators have reported that foot shock stress decreased NE concentration in the brain (Barchas and Freedman, 1963; Moore and Lariviere, 1964; Salama and Goldberg, 1969; Par and Livingston, 1970; Weiss et al., 1970; Fulginiti and Orsinger, 1971; Maynert and Levi, 1971; Baldessarini, 1972; Bliss et al., 1972; Thierry, 1972; and Shaliapina, 1973).

Immobilization stress also has been reported to decrease NE concentration (Corrodi, 1968; Par and Livingston, 1970; and Loginova, 1973) Heat or cold stress (Salama and Goldberg, 1969; Legrand et al., 1971; Brown and Van Huss, 1973; Eichelman and Thoa, 1973); crowding (Bliss and Ailion, 1969) swimming stress (Stone, 1970; Stolk et al., 1974); Pogodaev et al., 1970), and hypoxia (Debyadji et al., 1965; Perovic et al., 1969) also have been reported to decrease NE concentration.

NE turnover was found to be increased after foot shock stress (Bliss et al., 1968; Fulginiti and Orsinger, 1971; Huttunen, 1971; Baldessarini, 1972; Glowinski et al., 1972; Thierry, 1972; Stolk et al., 1974), after REM sleep deprivation (Mark et al., 1969), after fighting (Stolk et al., 1974;
Stolk et al., 1974b) after cold and heat stress (Simmonds, 1971; Clarke and Sampath, 1976) after immobilization (Fuxe et al., 1970) and after swimming (Stone, 1970).

However, in spite of the documentation of a decrease in NE level and an increase in NE turnover, other investigators have observed that stress increases NE level. For example, NE level has been reported to increase after foot shock stress (Welch and Welch, 1970), after immobilization (Par and Livingston, 1970), after heat or cold (Menon and Dandiga, 1969; Kohashi and Oka, 1973), and after fighting (Par and Livingston, 1970).

A decrease in NE turnover was found after hypoxia by Brown et al. (1974). Isolation resulted in a decrease in NE turnover according to Modigh (1974).

On the other hand, some authors have found no change in NE level or turnover after stress. For example, no change in NE level was found after foot shock stress by Fulginiti and Orsigher (1971), after heat or cold exposure (Gibson et al., 1969), after exercise (Goldberg and Salama, 1970), or after R.E.M. sleep deprivation (Radulovacki, 1973; Stern et al., 1971). No change in NE turnover was reported after cold, fighting and exercise (Gibson et al., 1969; Stolke, et al., 1974; Goldberg and Salama, 1970).

Several studies have focussed on the relationship between monoamine synthesis and adrenocorticotropic hormone (ACTH) secretion after exposure of animals to a stressful situation. Hedge et al. (1976) exposed rats to ether, formalin or cold stress, but found increases in the rate of $^{3}$H-NE and $^{3}$H-DA
accumulation after $^3$H-tyrosine only after ether stress. However, all three stressors increased ACTH secretion as indicated by plasma corticosterone levels. Dexamethasone treatment blocked the stress induced ACTH secretion, but had no effect on basal or stress induced rates of amine synthesis. The authors concluded that catecholamines participated in mediating ether stress induced ACTH secretion.

Hunger and stimulation of the lateral hypothalamic area were the "stressors" used by Torda (1976). He found large increases in the NE content of the hypothalamus in rats.

Recent studies have shown increases in brain NE level after drum stress (Goldberg and Salama, 1970), formalin injection (Carr and Moore, 1968), and noise stress (Moore and Lariviere, 1964).

Many of the observed changes in NE level were small. For example, Reid et al. (1968) found a 5% decrease in NE content in the whole brain of the rat after heat stress, while Ordy et al. (1966) found a 55% decrease in NE content after foot shock stress in the monkey; the amount of change was quite variable and seemed to depend on the type and severity of stress and the species used. There also is a difference with regard to the effects of stress given over a period of time. The variation in the biological response to the quantity of stress has not been studied as extensively as responses to acute stress. Electrolytic lesions destroying the locus coeruleus unilaterally immediately prior to foot shock in rats results in a prevention of the stress induced
reduction of NE in the cerebral cortex ipsilateral to the lesion, but not on the contralateral side (Korf et al., 1973). Also, a variety of psychoactive drugs prevent the reduction of NE in foot shock stressed (Kidbrink et al., 1972).

The effects of stress on central acetylcholine (ACh) and gamma amino-butyric acid metabolism have not been studied extensively. Sagales and Domino (1973) found no change in ACh concentration after R.E.M. sleep deprivation. Zajaczkowski (1975) found variable changes in ACh content in rat brain during foot shock stress and post-stress exhaustion. Pogodaev (1969) also found variable changes in ACh in distinct brain parts after exercise. Naik (1970) reported that fasting increased ACh and decreased acetylcholinesterase (AChE) activity. He also showed that stress of centrifugation decreased ACh content and increased AChE activity. Menon (1969) showed that heat stress produced a decreased ACh content.

Literature on the effects of stress on GABA also is quite scarce. Recently Seminginousky (1977) demonstrated an increase in cerebral GABA content during immobilization stress. In contrast Faiman et al. (1977) reported a decrease in GABA concentration during hyperbaric oxygenation.

Central tyrosine hydroxylase activity, monoamine oxidase activity, and tryptophan hydroxylase activity have been studied after stressful situations. (Palkovits et al., 1975; Kvetnansky et al., 1975; Pfeifer, 1974). The activity of these enzymes were reported to vary with respect to the
brain part studied. In brief, monoamine oxidase activity increased, tyrosine hydroxylase activity decreased, and tryptophan hydroxylase activity increased. These studies clearly suggest an involvement of central NE, 5-HT, ACh and GABA systems in the response to stress. The question remains, however, as to the nature, direction and importance of the roles of these distinct systems.

An overall picture of the central neural response to stress obviously is not clear. The changes in transmitter concentration and turnover vary, and there certainly is an interaction between the levels of circulating corticosterone and sex hormones, transmitter levels or metabolism. For example, foot shock decreases testosterone level (Bliss et al, 1968) and increases progesterone level (Ladisich, 1975). Hydrocortisone and norethynodrel both will decrease brain 5-HT level (Curzon, 1971, 1972).

Allucci (1977) found that ether stress caused a significant decrease in the concentration of hypothalamic NE, but had no effect on catecholamine concentrations in other regions. Pretreatment with betamethasone prevented the decrease in hypothalamic NE which was seen after ether stress. Betamethasone alone had no effect on midbrain NE, but pretreatment with betamethasone followed by ether stress increased midbrain NE.

Factors other than the adrenal-pituitary hypothalamic axis may be playing a role in stress as demonstrated by the experiments of Brown and Van Huss (1973). They found that when animals were forced to exercise they showed changes in NE and 5-HT centrally, while animals trained to exercise
for a reward failed to show such changes, even though the experimental conditions were similar.

It is clear from the above that there is an absence of agreement as to the concomitant or determinant changes in central monoamine levels or turnover during stress. Some possible explanations for the inconsistent results found in the literature include the use of different stressors, and the use of only one species, genera or strains of animal. These variables are emphasized in Table I. With the object of clarifying the inconsistencies in the field, the research described below focuses on the changes in concentration and/or turnover rate of four central neurotransmitters using four different stress inducing paradigms and two strains of mice and quantification of the foot shock stress. These experiments also focus on a range of behavioral parameters, including avoidance conditioning.

B. EXPERIMENTAL DESIGN

1. Effect of Foot Shock Stress on Whole Brain NE, 5-HT, ACh and GABA Level.

Fifty-six groups (n=5/group) of male CF-1 mice were used. The experimental groups (n=28) received 0, 10, 20, 30, 40, 50 or 60 shocks immediately prior to sacrifice. The control groups (n=28) were placed in the LVE shock chamber (p.14) then removed (without receiving any shocks) 0, 10, 20, 30, 40, 50 or 60 min later. Thus, the effects of increasing numbers of foot shocks on whole brain levels of NE,
TABLE I

STRESS AND NEUROTRANSMITTERS: SUMMARY OF THE LITERATURE

<table>
<thead>
<tr>
<th>TYPE STRESS</th>
<th>SPECIES</th>
<th>CONCENTRATION</th>
<th>TURNOVER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot shock</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Par and Livingston (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Thierry (1972)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Salama and Goldberg (1969)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Glownski et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>-</td>
<td></td>
<td>Baldessarini (1972)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Moore (1964)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Barchas and Freedman (1963)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Maynert and Levy (1971)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Weiss et al. (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Fulgini et al. and Orsinger (1971)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Glownski et al. (1972)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Baldessarini (1972)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Huttenen (1971)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Stol (1975)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Curzon (1974)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>0</td>
<td></td>
<td>Fulgini et al. and Orsinger (1971)</td>
</tr>
<tr>
<td>Immobilization</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Welch and Welch (1970)</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>-</td>
<td></td>
<td>Logniva (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Corrodi (1968)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Fuxe et al. (1970)</td>
</tr>
</tbody>
</table>

++: Increase; -:Decrease; 0: No Change.
<table>
<thead>
<tr>
<th>TYPE STRESS</th>
<th>SPECIES</th>
<th>CONCENTRATION</th>
<th>TURNOVER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>REM Sleep Deprivation</td>
<td>Rat</td>
<td>0</td>
<td>+</td>
<td>Mark et al. (1969)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>0</td>
<td></td>
<td>Radulovacki (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Stern (1971)</td>
</tr>
<tr>
<td>Heat</td>
<td>Rat</td>
<td>0</td>
<td>+</td>
<td>Gibson et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Menon and Dandiga (1969)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Legrand et al. (1969)</td>
</tr>
<tr>
<td>Cold</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Salama and Goldberg (1968)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Brown and Van Huss (1977)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>-</td>
<td></td>
<td>Eichelman (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td>+</td>
<td>Gibson (1969)</td>
</tr>
<tr>
<td></td>
<td>Rabbit</td>
<td>+</td>
<td></td>
<td>Clark and Sampath (1976)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Kohashi and Oka (1973)</td>
</tr>
<tr>
<td>Exercise</td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Goldberg and Salama (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Goldberg and Salama (1970)</td>
</tr>
<tr>
<td>Isolation</td>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>Modigh (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Stolk et al. (1974)</td>
</tr>
<tr>
<td>Fighting</td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Stolk et al. (1974)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Rat</td>
<td>-</td>
<td>-</td>
<td>Brown et al. (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Perovic et al. (1969)</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Bliss and Ailion (1969)</td>
</tr>
<tr>
<td>Swimming</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Stone (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>Stolk et al. (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Stone (1970)</td>
</tr>
</tbody>
</table>
### TABLE I (Continued)

**STRESS AND NEUROTRANSMITTERS: SUMMARY OF THE LITERATURE**

#### 5-HYDROXYTRYPTAMINE

<table>
<thead>
<tr>
<th>TYPE STRESS</th>
<th>SPECIES</th>
<th>CONCENTRATION</th>
<th>TURNOVER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot Shock</td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Ladisch (1974;1975)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Curzon (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>Salama and Goldberg (1969)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>Bliss et al. (1968)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Modigh (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Curzon (1974)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Bliss et al. (1968;1972)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>0</td>
<td></td>
<td>Baldessarini (1974)</td>
</tr>
<tr>
<td>Immobilization</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Curzon (1971;1972)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Heide and Peters (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Morgan et al. (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Bliss (1972)</td>
</tr>
<tr>
<td>REM Sleep Deprivation</td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Modigh (1974)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>+</td>
<td></td>
<td>Radulovacki (1976)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>-</td>
<td></td>
<td>Radulovacki (1976)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Stern et al. (1971)</td>
</tr>
<tr>
<td></td>
<td>Cat</td>
<td>0</td>
<td></td>
<td>Radulovacki (1973)</td>
</tr>
<tr>
<td>Heat</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Curzon (1971)</td>
</tr>
<tr>
<td></td>
<td>Guinea Pig</td>
<td>+</td>
<td></td>
<td>Francesconi and Maher (1974)</td>
</tr>
<tr>
<td>Cold</td>
<td>Rat</td>
<td>0</td>
<td></td>
<td>Gibson (1969)</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>+</td>
<td></td>
<td>Dixit and Buckley (1969)</td>
</tr>
<tr>
<td>TYPE STRESS</td>
<td>SPECIES</td>
<td>CONCENTRATION</td>
<td>TURNOVER</td>
<td>REFERENCE</td>
</tr>
<tr>
<td>------------------</td>
<td>---------</td>
<td>---------------</td>
<td>----------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Exercise</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Anakhina (1973)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td>0</td>
<td>Goldberg and Salama (1970)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>0</td>
<td>0</td>
<td>Goldberg and Salama (1970)</td>
</tr>
<tr>
<td>Isolation</td>
<td>Mouse</td>
<td>+</td>
<td></td>
<td>Welch and Welch (1970)</td>
</tr>
<tr>
<td>Fighting</td>
<td>Rat</td>
<td></td>
<td></td>
<td>Stolk et al. (1974)</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>Rat</td>
<td>-</td>
<td>+</td>
<td>Brown et al. (1974)</td>
</tr>
<tr>
<td>Aggregation</td>
<td>Rat</td>
<td></td>
<td></td>
<td>Bliss and Ailion (1969)</td>
</tr>
<tr>
<td>Food Deprivation</td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Curzon et al (1972)</td>
</tr>
</tbody>
</table>

(D)
### TABLE I (Continued)

**STRESS AND NEUROTRANSMITTERS: SUMMARY OF THE LITERATURE**

<table>
<thead>
<tr>
<th>TYPE STRESS</th>
<th>SPECIES</th>
<th>CONCENTRATION</th>
<th>TURNOVER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foot Shock</td>
<td>Mouse</td>
<td>+</td>
<td></td>
<td>Sobotka (1969)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>+</td>
<td>-</td>
<td>Zajoczkowska (1969)</td>
</tr>
<tr>
<td>Food Deprivation</td>
<td>Rat</td>
<td>+</td>
<td>-</td>
<td>Naik (1970)</td>
</tr>
<tr>
<td>Heat</td>
<td>Rat</td>
<td>-</td>
<td></td>
<td>Menon (1969)</td>
</tr>
<tr>
<td>Exercise</td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Pogodaev et al. (1969)</td>
</tr>
<tr>
<td>REM Sleep Deprivation</td>
<td>Mouse</td>
<td>0</td>
<td></td>
<td>Sagale and Domina (1972)</td>
</tr>
</tbody>
</table>

**ACHTYLCHOLINE**

<table>
<thead>
<tr>
<th>TYPE STRESS</th>
<th>SPECIES</th>
<th>CONCENTRATION</th>
<th>TURNOVER</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat</td>
<td>Rat</td>
<td>+</td>
<td></td>
<td>Semiginovsky (1977)</td>
</tr>
<tr>
<td>Hyperbaric Oxygen</td>
<td>Mice</td>
<td>-</td>
<td></td>
<td>Faiman (1977)</td>
</tr>
</tbody>
</table>

**GAMMA-AMINO-BUTYRIC ACID**

(E)
5-HT, Ach and GABA were obtained by comparing each experimental group to its appropriate control.

2. Time Course of the Effects of Foot Shock Stress on Whole Brain NE and 5-HT Levels.

Male CF-1 mice (n=120) were used to determine the time course of the changes in NE and 5-HT content seen in the first experiment. The stressed mice (n=60) received 60 foot shocks (1/min) in the LVE chambers. The control groups (n=15/group) spent 60 min in the shock chamber but were not shocked. Whole brain levels of NE and 5-HT were determined immediately and 1, 4 and 8 hr after the removal of the animals from the shock chambers.

3. Effect of Foot Shock on NE Concentrations in Brain Parts, on Whole Brain NE and Ach Turnover Rate, and on Whole Brain 5-HIAA Level.

a. Brain Parts

Male CF-1 mice (n=200) were used. The stressed mice received a total of 60 shocks in the LVE chambers. The control mice also spent 60 min in the shock chamber but were not shocked. Immediately following the 60th shock (or 60 min session) the mice were sacrificed and NE levels measured in 4 distinct brain regions: 1) telencephalon; 2) di- and mesencephalon; 3) cerebellum; and, 4) pons plus medulla. The dissection procedure is described in Chapter II (p.15).
b. Whole Brain ACh and NE Turnover Rate

Male CF-1 mice were used to determine the turnover rates of ACh (n=50) and NE (n=50). The experimental animals for each study (n=25) received a total of 60 shocks. Control mice (n=25) for each determination were placed in the shock chambers for 60 min but no shock was given.

Immediately following the 60th shock (or 60 min session) the mice were injected with $^3$H-choline or $^3$H-tyrosine and/or saline and whole brain ACh and NE turnover rates. The animals were after injection sacrificed at various times.

c. 5-HIAA Level in Whole Brain

Male CF-1 mice (n=50) were used to determine the 5-HIAA level in whole brain. The stressed mice (n=25) received a total of 60 shocks in the LVE chambers, while the control animals (n=25) spent 60 min in the chambers but were not shocked. Immediately following the 60th shock (or 60 min session) the mice were sacrificed and the 5-HIAA level determined (p.29).

C. RESULTS

In CF-1 mice, significant changes occurred in the levels of all four neurotransmitters; these changes depended on the number of foot shocks (30-60) administered (Fig. 3-6). It is important to note (Fig. 3-6) that the control groups (n=7/neurotransmitter studied) did not show variations as a function of the amount of time spent (1-60 min) in LVE chamber.
Whole brain NE level (Fig. 3) was decreased significantly following the administration of 30-60, but not 20 or less, shocks. Concomitantly, 5-HT level was elevated significantly (Fig 4). Likewise, ACh (Fig. 5) and GABA (Fig. 6) levels were higher. However, these small increases reached statistical significance for ACh only after 30 or 60 shocks, and for GABA only after 50 foot shocks.

Figure 7 represents the values for whole brain levels of NE and 5-HT immediately, 1, 4, and 8 hr after 50 foot shocks. As can be seen, the levels of NE and 5-HT returned to normal (pre-shock) levels by the fourth post-stress hour. The levels were still significantly changed ($p < 0.05$) 1 hr after shock. These results indicate the reversibility of the effect of stress on monoamine levels.

The effects of stress on NE level in brain parts, NE and ACh turnover rates in whole brain, and 5-HIAA level in whole brain are summarized in Table II. A significant decrease in NE was seen only in the di-mesencephalon ($p < 0.05$). The whole brain turnover rate of ACh was significantly decreased ($p < 0.02$), while NE turnover was significantly increased ($p < 0.05$) following stress. Surprisingly, foot shock stress did not significantly affect 5-HIAA level. This latter finding may be due to the large degree of variance obtained.

D. DISCUSSION

The results indicate that long duration (30-60) but not short duration (20 shocks) stress significantly affects central neuro
Figure 3. Effect of consecutive foot shocks on whole brain norepinephrine (NE) level. Significant difference from control appeared after 30 shocks. NE values were corrected for 100% recovery. Probabilities based on Student's t-test for independent means. Shocked mice were compared with appropriate control animals. Each value (± standard error of the mean, SEM) represents 10 mice. *: p<0.05; **: p<0.01.
Figure 4. Effect of consecutive foot shocks on whole brain 5-hydroxytryptamine (5-HT) level. Significant differences from control appeared after 30 shocks. *: p<0.05; **: p<0.01. See Fig. 3 for additional details.
Figure 5. Effect of consecutive foot shocks on whole brain acetylcholine (ACh) level. Significant differences from control appeared after 30 shocks. *: p<0.05; **: p<0.02. For added details see Fig.3.
Figure 6. Effect of consecutive foot shocks on whole brain gamma-aminobutyric-acid (GABA) level. A significant ($p < 0.05$) increase was seen only in the group which received 50 shocks. See Fig.3 for additional details.
Figure 7. Time course for the normalization of whole brain norepinephrine (NE) level after cessation of foot shock stress. The stress induced significant decrease ($p < 0.01$) in NE concentration was still observable 1 hr. after termination of shock. However, the experimental and control groups did not differ significantly either 4 or 8 hr. post-stress. Each value ($\pm$SEM) represents 10 mice. Probabilities based on Student’s t-test for independent means. *:$p < 0.05$; **:$p < 0.01$. 
Figure 8. Time course for the normalization of whole brain 5-hydroxytryptamine (5-HT) level after cessation of foot shock stress. The significant increase in 5-HT produced by stress also was observed 1 hr. after termination of foot shock. Four and eight hours after termination of shock, the experimental and control groups did not differ significantly. *: p < 0.05. See Fig. 7 for additional details.
TABLE II. Effect of stress on norepinephrine (NE) levels in three brain parts, acetylcholine (ACh) and NE turnover rate (T.R.) in whole brain, and 5-hydroxyindole acetic acid (5-HIAA) level in whole brain. A t-test revealed that a significant (p<0.05) decrease in NE occurred in the mesencephalon (MD), but not in the telencephalon (T), cerebellum (Cere) or pons-medulla (PM). Twenty five brain parts were used for each determination. Whole brain ACh turnover was significantly (p<0.02) decreased, while NE turnover was significantly (p<0.02) increased. Each value (+ SEM) represents 25 mice. Shock stress did not significantly change whole brain 5-HIAA level. The data for NE concentration in brain parts and 5-HIAA content in whole brain are presented as nanogram per gram (ng/g) wet brain weight. The values are corrected for 100% recovery. The data for NE T.R. are presented in nanograms per gram per hour (ng/g/hr), while the ACh T.R. is presented in nanomoles per gram per hour (nM/g/hr). Table on following page.
### Table II

**EFFECT OF STRESS ON NE LEVEL IN BRAIN PARTS, ACH AND NE TURNOVER RATE IN WHOLE BRAIN, AND 5-HIAA LEVEL IN WHOLE BRAIN**

<table>
<thead>
<tr>
<th>Brain Parts</th>
<th>NE (ng/g) ± SEM</th>
<th>ACH T.R. ± SEM (nM/g/hr)</th>
<th>NE T.R. ± SEM (ng/g/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T</td>
<td>MD</td>
<td>Cere</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>102 ± 31</td>
<td>452 ± 31</td>
<td>98 ± 21</td>
</tr>
<tr>
<td><strong>FOOT SHOCK</strong></td>
<td>108 ± 16</td>
<td>310 ± 26</td>
<td>112 ± 18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>5-HIAA (ng/g) ± SEM</th>
<th>whole</th>
<th>brain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CONTROL</strong></td>
<td>321 ± 98</td>
<td></td>
</tr>
<tr>
<td><strong>FOOT SHOCK</strong></td>
<td>386 ± 44</td>
<td></td>
</tr>
</tbody>
</table>

Details on preceding page.
transmitter metabolism, suggesting that these systems play an important role in an organism's "coping" response. Whole brain NE level decreased significantly (Fig. 3). These effects appear to be due to changes in NE metabolism principally in di- and mesencephalon (Table II). A concomitant elevation in whole brain 5-HT level also was observed (Fig. 4). Although 5-HIAA level (Table II) was not significantly changed, possibly because of the variance obtained, the stressed group did evidence a 20% increase in brain 5-HIA content. Likewise, brain ACh and GABA levels showed small but consistent increases (Fig. 5 and 6) while ACh turnover (Table II) was reduced (20%). Thus, severe stress appears to increase central NE and 5-HT turnover, to decrease ACh turnover, and have only a small effect on GABA concentration. These changes, furthermore, appear to be temporary, as NE and 5-HT brain concentrations return to normal 4 hr, but not 1 hr post-stress (Fig. 7 and 8).

The decrease in NE level after foot shock stress (30 + shocks), in general, is in agreement with several other studies concerning the effect of similar types of stress on brain NE concentration. In general, NE in most brain regions studied decreases with acute stress, but shows no change or an increase if the same stress is repeated or given chronically (Huttunen, 1971; Nielson and Fleming, 1977; Welch and Welch, 1965; and, Weiss et al., 1970). According to Stone (1975), the changes in amine content, at least the reduction in NE level during acute stress, are
most likely the result of increased NE turnover. It has been shown that barbiturates (Maynert and Levi, 1964; Lidbrink et al., 1972), benzodiazepines (Taylor and Laverty, 1969), and meprobamate (Lidbrink et al., 1972) prevent the reduction in brain NE associated with foot shock stress in rats. However, Welch and Welch (1970) consistently have found increases in NE after stress, and argue that stress increases neuronal activity since increased synthesis is usually considered to be secondary to increased release of NE. Weiner (1970) suggested a short term regulation of NE by a negative feedback mechanism in which increased NE utilization depletes a small intraneuronal pool. Presumably, it is the function of this pool to inhibit tyrosine hydroxylase. Thus, under stressful conditions, when NE is being released, tyrosine hydroxylase is disinhibited. Welch and Welch (1970) suggested that MAO may be inhibited during stress. Kobayashi (1975) found that tyrosine hydroxylase activity was increased selectively in the arcuate nucleus, but not in the median eminence, during stress. Tyrosine hydroxylase activity has been reported unchanged in whole brain (Gibson et al., 1969) and in the hypothalamus (Stone, 1973) after stress. The finding that exposure to cold stress increased tyrosine hydroxylase activity in the medulla oblongata, but not in the hypothalamus, was interpreted as reflecting an increased CA synthesis in the brain stem but not in the hypothalamus. Newly synthesized NE appears to be preferentially used in the brain stem of rats stressed by foot shock (Thierry et al., 1970).
Palkovits et al. (1975, 1976) found that 5-HT concentration and tryptophan hydroxylase activity were elevated in the dorsal raphe nucleus after immobilization stress. Stress did not alter enzyme activity in any other brain region studied by Palkovits et al. (1975). Vermes et al. (1973) used cold, restraint, formalin and ether as stressors. Only ether stress caused an increase in 5-HT concentration up to 30 min post-stress, then a decrease (up to 2 hr post-stress). Plasma corticosteroids showed the opposite effects, namely a decrease followed by a rapid increase which was inversely proportional to the 5-HT content of the hypothalamus. Telegdy (1975) demonstrated that 5-HT content after foot shock increased in the hypothalamus 30 min after shock and in the mesencephalon 60 min post-shock. The 5-HIAA content increased in parallel with the elevated 5-HT content. Since the increase in 5-HT was preceded by an increased plasma corticosterone level, it was suggested that the restoration of 5-HT in brain was in part due to an effect of plasma corticosterone. It has been reported that increased corticosterone levels are correlated with an increase in 5-HT level in the hypothalamus (Telegdy, 1974).

Results of the present study could be explained in terms of a number of mechanisms. For instance, increases in 5-HT may be due to an inhibition of MAO. On the other hand, MAO inhibition also would increase the level of NE, probably in all brain regions. However, if tyrosine hydroxylase inhibition has preceded MAO inhibition, as postulated by Leblanc (1976), a decrease in NE level and an increase in
5-HT levels would be expected. There is evidence that a decrease or increase in cholinergic activity leads to an inhibition or activation of adrenergic neurons (Anden et al. 1970; Bartholini and Pletscher, 1971; Hitzenman et al. 1972). It seems likely that striatal DA and cholinergic neurons are functionally interconnected and mutually regulating (Lloyd et al. 1973). Along these some lines, evidence has been provided for nigrostriatal projections originating from the large cells of pars compacta of substantia nigra. In addition, fibers from striatum have been shown to project to substantia nigra terminating mainly in pars reticulata (Fonnum et al., 1974). Dopaminergic innervation originating from substantia nigra impinges on numerous cholinergic neurons intrinsic to striatum (Butcher and Butcher, 1974). Available evidence suggests that in vivo the rate of dopamine release inhibits the effects and the turnover rate of ACh (Anden, 1971). An important control in the activity of DA cell bodies is the feedback loop of GABAergic neurons described by McGeer et al., 1975. GABA participates at two levels in the biochemical operation of striatum: it is released by small interneurons for local control of excitability and it carries messages initiated in striatum to globus pallidus (McGeer et al., 1975) and substantia nigra (Fonnum et al., 1974).

Our observation that GABA concentration was increased as a result of stress was, as with ACh, a novel finding.
However, this increase in GABA level reached statistical significance only in the group of mice which received 50 shocks. Interestingly, a dopaminergic-cholinergic-GABA-ergic loop has been posited in the feedback control of mesencephalic DA neurons (Roberts, 1976).

The functional significance of the stress induced changes in neurotransmitter metabolism observed in the present study is not clear. The following question was therefore asked: what would happen if our stressed animals had some control over the stressor? Would the neurotransmitters change under conditions in which the animals had the opportunity to escape the stressful situation or stimulus? Having ascertained that NE, 5-HT and ACh levels or turnover in the brain change during stress, experiments were designed to demonstrate what, if any, functional or behavioral significance such neurochemical changes have.
CHAPTER IV

LEARNING AND NEUROCHEMICAL VARIABLES

A. INTRODUCTION

The effect of learning on brain NE, 5-HT, and ACh metabolism has been studied by many investigators who used a number of behavioral and biochemical paradigms.

Results have been somewhat contradictory and inconclusive. The issue of separating the effects of nonlearned variables, such as motivation and motor ability from learning factors, such as retention, retrieval and consolidation, is always present. The dissection of these variables always must be considered in any study on learning (Wright, 1974).

The bulk of the literature on neurotransmitters and learning has employed the empirical approach of using the level or turnover of the transmitter under study as the independent variable, manipulating it, then looking for subsequent changes in behavior. This approach will be discussed below in Chapter VI (p. 86). A second approach involves using the organism as an independent variable, manipulating it, then measuring subsequent changes in transmitter level and turnover.

A study by Fuxe and Hanson (1967) demonstrated that avoidance behavior, but not the stress of foot shock, caused an increase in brain NE turnover as measured by an increase in the number and intensity of fluorescing NE cell bodies and
terminals throughout the brain. Other studies reported similar results. Fulginiti and Orsingher (1971) demonstrated a 24-143% increase in $^3$H-NE specific activity in the hypothalamus after avoidance training. There was no effect of avoidance conditioning on 5-HT levels in either the hypothalamus or cerebral cortex. Weiss et al., (1970), found that animals permitted to escape shock showed an increase in brain NE level.

Enhanced NE synthesis after positively reinforced behavior was suggested by the work of Lewy and Seiden (1972). They reported a 25% increase in the specific activity of $^3$H-NE in the brainstem and diencephalon after bar-pressing for water. There was no effect of water deprivation on regional brain NE concentration. Yuwiler and Olds (1973) demonstrated that rats which lever pressed for rewarding hypothalamic stimulation showed a decrease in the level of hypothalamic NE and whole brain DA after a 20 min test session. Animals which were given brain stimulation by the experimenter did not show such changes in catecholamine concentrations. These results suggest that the emission of a learned response is associated with an increase in central NE turnover.

Results of these various studies are difficult to interpret since, as mentioned above, factors other than learning could modify or interfere with the effects of a conditioned response on neurotransmitter level or turnover. For instance, motor activity may have been enhanced and the changes in NE may have been due, therefore, to an increase in muscular activity.
The effects of a conditioned response on brain 5-HT and ACh levels and turnover have been studied by a few investigators (Karczmar, 1977). Matthies et al., (1974) looked for changes in the ACh content of different brain regions of the rat during a learning experiment. They found in the hippocampal region the free acetylcholine was highly increased immediately after the training whereas the stable bound ACh and labile-bound fraction rose 70 min. and 4 hrs. respectively after completion of training. Only small alterations of the ACh fractions were observed in the visual and auditory cortices. In the corpus striatum no changes appeared. The concluded the changes observed in ACh content during and after training seem to be of great significance at the initial stages of memory formation. The hypothetical involvement of these neurotransmitters in learning has been derived from pharmacological studies and behavioral studies (Karczmar, 1976).

In the experiments presented in Chapter III, changes in brain NE, 5-HT and ACh were found to occur after foot shock stress. The experiments reported below were designed to determine whether such changes would be seen in animals which were given the opportunity to escape or avoid foot shock stress.

B. EXPERIMENTAL DESIGN

Effect of Acquisition of a Conditioned Avoidance Response (CAR) on NE, 5-HT, and ACh levels.
Whole brain NE, 5-HT and ACh levels were determined in five groups of CF-1 mice:

Group 1: Control Group A (CG-A):
These mice (n=60) were placed in the starting base chamber (Al, Fig. 2) of the climbing screen, then sacrificed 60 min later.

Group 2: Control Group B (CG-B):
These mice (n=60) were placed in the Lehigh Valley Electronics (LVE) stress chambers for 60 min then sacrificed. No shock was administered.

These mice (n=60) were exposed to a series of 50 unavoidable 2 sec shocks (1/min) after placement in the climbing screen starting base chamber (Al, Fig. 2). The mice were sacrificed immediately after the final shock.

Group 4: Shock Stressed Group A (SG-B):
These mice (n=60) were placed in the LVE chambers and administered over a 50 min period a mean of 25 unavoidable shocks prior to sacrifice. The frequency and duration of these shocks were reduced systematically over the 50 min session. Thus, Group 4 received approximately the same number and duration of shocks as the conditioned avoidance group (CAR) (group 5) see below. The frequency and duration of the shocks received by mice in this latter group (CAR group) decreased as the experiment progressed. Consequently, the pattern, number and duration of shocks were recorded during an avoidance session of group 5.
The mice in group 4 subsequently were given similar parameters of shock except that the mice of group 4 were not allowed to escape. This design allowed for the possibility that changes occurring in the stressed animals (Group 3) were due to the fact that the SG-A mice received more shocks and were restricted in their movements by the small climbing screen base chamber as compared to the large LVE chamber.

Group 5: Conditioned Avoidance Group (CAR):

These mice (n=60) received 50 avoidance conditioning trials using the climbing screen apparatus and procedure (Chapter II). These mice were sacrificed immediately after training.

C. RESULTS

The animals in Group 5 (CAR) evidenced a progressive and significant decrease in the latency for leaving the base chambers. By the 25th trial these latencies averaged less than 5 sec, indicating acquisition of a conditioned avoidance response (CAR). Acquisition of the CAR, however, was not associated with any significant effects on whole brain levels of NE, 5-HT or ACh (Fig. 9) as compared to the control groups (1 and 2), which themselves did not differ. There were significant differences, on the other hand, in the whole brain concentrations of these neurotransmitter substances in the stressed groups, which themselves did not differ, when compared with the other 3 groups (1, 2, and 5).
The results indicate that 1) acquisition of a conditioned avoidance response (CAR) does not affect whole brain NE, 5-HT or ACh concentrations, and 2) stress results in a decrease in whole brain NE level and an increase in central 5-HT and ACh content, if the animals are not given an opportunity to escape or avoid the stressful stimuli. These findings are in agreement with those reported in Chapter III, but are in discordance with those of other investigators who found changes in brain neurotransmitter levels subsequent to the acquisition of a CAR.

Studies by Weiss et al. (1970, 1975) are relevant. They performed an experiment in which rats received electric shock through tail electrodes. Two rats were wired in series so that both received the identical intensity and duration of tail shock (impedance was similar in both rats). One animal was allowed to escape the shock by turning a wheel. This wheel turning stopped the shock for both animals. Weiss et al. (1970) found that animals which could escape the shock by turning a wheel showed an increased brain level of NE, while the "yoked" animals with no control over the shock showed a reduction in central NE content. Control over the shock, thus, seems important in determining the effect of both foot and tail shock induced changes in brain NE concentration.

Other investigators (Fuxe and Hanson, 1967; Lewy and Seiden, 1972; Yuwiler and Olds, 1973) have reported that
Figure 9. The effects of avoidance conditioning (CAR) and foot shock stress on whole brain norepinephrine (NE), 5-hydroxytryptamine (5-HT) and acetylcholine (ACh) levels. Control group A (CG-A); Control group B (CG-B); Shock stressed group A (SG-A); Shock stressed group B (SG-B); Conditioned avoidance group (CAR). Acquisition of the CAR was not associated with any significant effects on whole brain levels of NE, 5-HT or ACh as compared to control groups CG-A or CG-B, which themselves did not differ. Compared with the other 3 groups there were significant differences in NE, 5-HT and ACh in the stressed groups SG-A and SG-B. These latter two groups did not differ significantly. Each value (±SEM) represents 60 mice. Probabilities based on Student's t-test for independent means. *: p<0.01.
NE turnover is changed following the acquisition or performance of conditioned behavior. Our results agree with those of Fulginiti and Orsingher (1971) with regard to 5-HT level, but are in disagreement with these and the above investigators with regard to changes in central NE. The discrepancies may be due to the fact that Lewy and Seiden (1972) and Yuwiler and Olds (1973) used positive reinforcement behavioral paradigm versus our conditioned avoidance task. Also Weiss et al. (1970) used escape behavior rather than avoidance. Our results, on the other hand, agree with those of Weiss et al. with regard to the stress effect (no escape results in a decrease in NE). Therefore, it is possible that inescapable shock, escapable shock and avoidable shock produce different effects on central NE level, that is, a decrease, an increase and no change, respectively.

Assuming its importance in the regulation of subsequent behavior, stress should either facilitate or attenuate various behavioral routines. Stress may be acting to increase the activity of central catecholaminergic systems, and to decrease the activity of the cholinergic and serotonergic systems. In so doing, this activation, inhibition, or combination thereof, allows for, or in some way modulates, behavioral routines until an appropriate stress relieving response is found through trial and error. When this coping response is achieved and execution of "correct" behavioral sequences is accomplished, then the increased or decreased activity of select neurotransmitter systems (as measured by changes in concentration or turnover) are normalized.
If the neurotransmitter fluctuations which were found in these experiments have functional significance, then similarly induced changes in neurotransmitter activity, reflected as a decrease in NE and increases in 5-HT and ACh concentration, should produce predictable behavioral effects. If the stress-induced changes in brain transmitter systems have adaptive value for coping behavior, then stress should enhance the acquisition of avoidance conditioned behavior.

In order to test this hypothesis the experiments described below (Chapters V-IX) were designed to determine 1) the effect of stress on the acquisition of an avoidance conditioned response; 2) the effects on avoidance conditioning of drugs which mimic the "stress syndrome" neurochemically; 3) the relation between genetically determined endogenous levels of NE, 5-HT and ACh and avoidance conditioning; and, 4) the effect of stressors other than foot shock on conditioned avoidance acquisition and locomotor activity.
C. RESULTS

The animals in Group 5 (CAR) evidenced a progressive and significant decrease in their latency to leave each base chamber. By the 25th trial these latencies averaged less than 5 sec, indicating acquisition of a conditioned avoidance response (CAR). Acquisition of the CAR, however, was not associated with any significant effects on whole brain levels of NE, 5-HT or Ach (Fig. 9) as compared to the control groups (1 and 2), which themselves did not differ. There were significant differences, on the other hand, in the whole brain concentrations of these neurotransmitter substances in the stressed groups, which themselves did not differ, when compared with the other 3 groups (1, 2 and 5).

D. DISCUSSION

The results indicate that 1) acquisition of a conditioned avoidance response (CAR) does not affect whole brain NE, 5-HT or Ach concentrations, and 2) stress results in a decrease in whole brain NE level and an increase in central 5-HT and Ach content, if the animals are not given an opportunity to escape or avoid the stressful stimuli. These findings are in agreement with those reported in Chapter III, but are in discordance with those of other investigators who found changes in brain neurotransmitter levels subsequent to the acquisition of a CAR.

Studies by Weiss et al. (1970, 1975) are relevant. They performed an experiment in which rats received electric shock through tail electrodes. Two rats were wired in series
so that both received the identical intensity and duration of tail shock (impedance was similar in both rats). One animal was allowed to escape the shock by turning a wheel. This wheel turning stopped the shock for both animals. Weiss et al. (1970) found that animals which could escape the shock by turning a wheel showed an increased brain level of NE, while the "yoked" animals with no control over the shock showed a reduction in central NE content. Control over the shock, thus, seems important in determining the effect of both foot and tail shock induced changes in brain NE concentration.

Other investigators (Fuxe and Hanson, 1967; Lewy and Seiden, 1972; Yuwiler and Olds, 1973) have reported that NE turnover is changed following the acquisition or performance of conditioned behavior. Our results agree with those of Fulginiti and Orsingher (1971) with regard to 5-HT level, but are in disagreement with these and the above investigators with regard to changes in central NE. Possible explanations for these discrepant results include the use of a positive reinforcement behavioral paradigm by both Lewy and Seiden (1972) and Yuwiler and Olds (1973) versus our conditioned avoidance task. Also, Weiss et al. (1970) used escape behavior rather than avoidance. Our results, on the other hand, agree with those of Weiss et al. with regard to the stress effect (no escape results in a decrease in NE). Therefore, it is possible that inescapable shock, escapable shock and avoidable shock produce different effects on central NE level, that is, a decrease, an increase and no change, respectively.
Assuming its importance in the regulation of subsequent behavior, stress should either facilitate or attenuate various behavioral routines. Stress may be acting to increase the activity of central catecholaminergic systems, and to decrease the activity of the cholinergic and serotonergic systems. In so doing, this activation, inhibition, or combination thereof, allows for, or in some way modulates, behavioral routines until an appropriate stress relieving response is found through trial and error. When this coping response is achieved and execution of "correct" behavioral sequences is accomplished then the increased or decreased activity of select neurotransmitter systems (as measured by changes in concentration or turnover) are normalized.

If the neurotransmitter fluctuations which were found in these experiments have functional significance, then similarly induced changes in neurotransmitter activity, reflected as a decrease in NE and increases in 5-HT and Ach concentration, should produce predictable behavioral effects. If the stress induced changes in brain transmitter systems have adaptive value for coping behavior, then stress should enhance the acquisition of avoidance conditioned behavior.

In order to test this hypothesis the experiments described below (Chapters V-IX) were designed to determine 1) the effect of stress on the acquisition of an avoidance conditioned response; 2) the effects on avoidance conditioning of drugs which mimic the "stress syndrome" neurochemically; 3) the effect of genetically determined endogenous levels of NE, 5-HT and Ach on avoidance conditioning; and, 4) the
effect of other stressors on conditioned avoidance acquisition and locomotor activity
CHAPTER V

LEARNING AND STRESS

A. INTRODUCTION

The behavioral consequences of stress has been studied by a number of investigators. These behaviors include aggression (Valzelli, 1972), sex (Morris, 1954; Goldfoot and Baum, 1972) and ingestion (Antleman and Caggiula, 1977). Most studies report an increase in consummatory behavior due to a variety of stressors.

An interesting approach to the study of stress related behavior was described by Antelman et al., (1975). Their experimental manipulation involves the application of mild pressure to the tail of rats or mice. The "tail-pinch-induced behavior" reported by these investigators is quite dramatic. For example, eating in food-sated animals was found in over 95% of the rats tested. These investigators and others (Eichelman et al., 1976; Connor, 1971; Williams, 1971) reported findings which can be grouped into three categories: 1) the induction of a particular behavior, for example, eating; 2) exaggeration of lesion or pharmacologically induced behaviors; and, 3) recovery of normal behaviors which have been depressed as a consequence of physical or pharmacological treatment. The involvement of the central noradrenergic and serotonergic systems in the tail-pinch-induced
behaviors was strongly suggested by 1) the ability of \( \alpha \)-MPT, 5-HTP and tryptophan, to attenuate the responses induced by tail pinching, and 2) the ability of 6-OHDA injected into the substantia nigra region to produce deficits in both the onset and maintenance of tail-pinching-induced-behavior.

As suggested earlier, the behavioral response of an organism to stress may actually serve to reduce the adverse psychological and biochemical consequences of such stress. Reinstatement of homeostasis has been demonstrated by a number of investigators. For example, Stolk et al. (1974) demonstrated that shocked solitary rats showed increases in NE turnover, while rats shocked in the presence of another rat, against which aggression was possible, showed no such change.

The experiments presented in this section were designed to test the hypothesis that stress places an organism in a "readiness" posture, and allows more readily for the expression of successful trial and error behavior. Thus, stress should facilitate the acquisition of conditioned avoidance response.

B. EXPERIMENTAL DESIGN

1. Effects of Stress on Acquisition of a Conditioned Avoidance Response (CAR)

The acquisition of a CAR was studied in 2 groups of male CF-1 mice. Group 1: The mice in this group \( n=60 \) received a total of 60 unavoidable shocks \( 1/\text{min} \) in the Lehigh Valley (LVE) shock chambers as described in the methods section (Chapter
Group 2: The mice in this group (n=60) were placed in the shock chambers for 60 min. without being shocked. Immediately after removal from the LVE chambers, the mice were placed in the "climbing screen" and their acquisition of a CAR studied.

2. Effects of CAR on Stress-Induced Changes in Brain Transmitter Levels.

The effects of CAR training on stress-induced changes in whole brain NE and 5-HT concentrations were investigated in 3 groups of mice, each of which was subdivided into 3 subgroups (n=10/subgroup) based on the amount of time (0, 25 or 50 min) they spent in the climbing screen.

Group A (Controls): These animals (n=30) were not shocked or trained to avoid, but spent 60 min in the LVE chamber. The animals then were sacrificed for brain monoamine assay, after spending 0, 25 or 50 min in the initial base chamber of the climbing screen (Al, Fig 2).

Group B (Stressed): These mice (n=30) received 60 shocks in the LVE chamber, then were sacrificed after spending 0, 25 or 50 min in the initial climbing screen (Al) base chamber. No CAR training was given.

Group C (Stress + CAR): The 3 subgroups of mice comprising this group (n=30) received 60 unavoidable 2 sec shocks (1/min) in the LVE chambers, and then 0, 25 or 50 min
of avoidance conditioning in the climbing screen prior to sacrifice for central monoamine analysis. Note that the Group C 0-min subgroup (n=10) received the same treatment as the Group B 0-min subgroup (n=10).

C. RESULTS

The effects of stress on avoidance conditioning are presented in Fig 10. An analysis of variance (ANOVA) indicated that shock-stress facilitated the acquisition of the CAR. The ANOVA revealed a significant trial effect (p<.003) and treatment (p<0.01) effect. The interaction term (interaction between time and trials) was not sig.

The amount of time the stressed animals spent in the base chambers was less on every trial as compared to controls. Individual between group comparisons revealed significant differences after 20 or more trials. The stressed mice also totaled more CAR's than the control animals (Fig.10). There was no significant difference in climbing time (the amount of time it takes an animal to climb from one base chamber to the next) between the stressed animals and controls (Fig.11). The climbing time is a good indication of motor activity and coordination, since an animal with a motor deficit would spend more time going up the ramp from one base chamber to the next, whereas an animal with increased motor ability or activity may ascend the ramp faster. The similar climbing times for the controls and stressed animals suggest, therefore, that stress had no effect on
Figure 10. Effect of foot shock stress on subsequently conditioned avoidance behavior. Horizontal hatched line indicates that a latency of less than 5 sec. spent in the base chamber led to a successful avoidance response. An analysis of variance indicated a significant (p<0.001) treatment effect. A t-test revealed significant between group differences after 20, 25, 35, 45 (p<0.05) and 50 (p<0.01) trials, indicating that prior foot shock stress facilitates acquisition of a conditioned avoidance response. Inset: Total conditioned avoidance responses (CAR) emitted during the session. A significant (p<0.05) increase in the total number of CAR's emitted was seen due to stress. Each value (±SEM) represents 10 mice.
Figure 11. Effect of prior foot shock stress on the climbing ability (measured as climb time) of mice in the avoidance conditioning "climbing screen". An analysis of variance revealed a significant (p<0.001) trial effect, but no significant treatment effect. Thus, stress had no effect on the ability of the mice to climb the ramp. However, both groups demonstrated a decrease in climbing time as the experiment progressed, and as the animals evidenced acquisition of the avoidance response. Each value (±SEM) represents 10 mice.
motor activity per se. The effects of subsequent avoidance conditioning on the stress-induced changes in brain levels of NE and 5-HT are presented in Fig. 12. Avoidance conditioning attenuated the effect of stress on brain 5-HT but not NE level.

D. DISCUSSION

The results are consistent with our hypothesis that stress-induced changes in central neurotransmitter concentrations affects subsequently learned behaviors in mice, as foot shock stress facilitated the subsequent acquisition of a conditioned avoidance response (CAR). Avoidance conditioning, furthermore, reduced the shock induced change in central 5-HT, but not NE concent. Avoidance conditioning by itself did not, in our hands, affect brain neurotransmitter concentrations (Chapter IV). This result was not consistent with the findings of others (Fuxe and Hanson, 1967; Fulgini and Orsingher, 1971). As suggested earlier (Chapter IV) our findings with regard to avoidance conditioning effects on monoamines may differ as a result of our use of a different learning paradigm.

If the stress induced changes in neurotransmitter systems, measured as alterations in brain concentrations, are important in facilitating the acquisition of a CAR, them pharmacological manipulations of neurotransmitter concentrations in a similar manner should facilitate CAR acquisition. The experiments reported in the following chapter were designed to determine what effects drugs, which alter neurotransmitter metabolism in distinctly different ways, have on avoidance conditioning.
Figure 12. Effects of avoidance conditioning on stress-induced changes in whole brain 5-hydroxytryptamine (5-HT) and norepinephrine (NE) levels. Three groups were used: 1) a non-stressed control group which spent 60 min. in the LVE shock chamber, and 0, 25 or 50 min. in the climbing screen base chamber without receiving foot shocks or avoidance training at any time. 2) a stressed group (stress) which received 60 shocks (1/min.) in the LVE chamber, then were exposed to the climbing screen for 0, 25 or 50 min. without avoidance training; and, 3) a stress plus avoidance conditioning group (stress + CAR) which received 60 shocks in the LVE chamber, and then 0, 25 or 50 min. of avoidance conditioning in the climbing screen.

Monoamine concentrations were determined for each group at each of the 3 time intervals following completion of the stress, and expressed as percent differences from non-stressed control. A total of 9 subgroups (n=10 in each), thus, were analysed. The 5-HT and NE levels of the stress, and stress + CAR, groups were significantly higher and lower, respectively, than the control group at each of the 3 intervals (that is, after 0, 25 or 50 CAR trials). On the other hand, a t-test revealed a significant (p<0.05) decrease in 5-HT in the stress + CAR group as compared to the stress group, suggesting that avoidance conditioning attenuated the effect of stress on brain 5-HT level. In contrast, the absence of a significant difference between the stress and stress + CAR groups, with regard to whole brain NE levels, suggests that subsequent avoidance conditioning does not influence stress induced changes in NE concentration. Figure on following page.
Figure 12. Effects of avoidance conditioning on stress-induced changes in whole brain 5-hydroxytryptamine (5-HT) and norepinephrine (NE) levels. Details on previous page.
CHAPTER VI
DRUGS, NEUROCHEMISTRY AND LEARNING

A. INTRODUCTION

Two methods have been used to study the relationship between learning and neurochemistry. One approach uses neurotransmitter level as the independent variable, while the second employed the organism as the independent variable. In other words, in the first case the effects of altered neurotransmitter metabolism on learning processes, was evaluated while in the other case the effects of learning on transmitter concentration and/or turnover were measured. The possible involvement of the catecholaminergic, serotonergic and cholinergic systems in learning processes will be reviewed.

1. Catecholamine Systems

The effects of pharmacological manipulations on learning often depend on the physical requirements of the task involved. Often a change in learning behavior is attributed to a drug which impairs motor performance, thereby clouding the issue of causality and making correlative studies difficult to interpret. Impairment of avoidance acquisition may be due to alterations in motivation, discrimination and
performance, rather than learning processes per se. For example, treatment with \(\alpha\)-MPT does not affect the acquisition of a shuttlebox avoidance task if motor activity is not affected (Ahlenius, 1973).

In general, experimental procedures which decrease central catecholaminergic activity impair learning. Intraventricular or intercerebral administration of the catecholamine neurotoxin, 6-hydroxydopamine (6-OHDA), for example, has been shown to impair the acquisition of a conditioned avoidance response (CAR) (Cooper et al., 1973; Howard et al., 1974; Fibiger, et al., 1976). However, Cooper et al. (1973) has demonstrated that acquisition of an escape-avoidance response can be facilitated or impaired after 6-OHDA injections depending on the dose; doses which preferentially depleted NE to a greater extent that DA facilitated acquisition, while doses which depleted DA to a greater extend than NE either had no effect or impaired acquisition. Altogether, related the facilitation of acquisition to increased motor activity.

That the catecholamine systems are involved in learning also has been suggested by studies in which electrolytic lesions were made in the locus coeruleus of rats (Anlezark et al., 1973). Such lesions resulted in the impairment of a hunger-motivated response, but did not decrease motor activity. The use of alpha and beta adrenergic blocking agents have provided evidence that both types of adrenergic receptors are involved in the learning process (Vasquez et al., 1967; Merlo & Izquierdo, 1965). Disulfiram, a dopamine-beta-hydroxylase inhibitor has been shown to impair performance of an avoidance
response (Krantz and Seiden, 1968).

Direct placement of NE into the brain had an effect on a CAR which depended on the site of injection. Thus, when NE was given intraventricularly, CAR acquisition was facilitated, while NE injections directly into the reticular formation, impaired CAR acquisition (Wise et al., 1973).

Stein (1969) and coworkers (Stein and Wise, 1970; Stein et al., 1975) have suggested that the facilitatory effect on learning which follows treatments which enhance adrenergic activity is mediated by a specific alpha-adrenergic reinforcement system. Accordingly, stimulation of this system enhances consolidation by increasing the probability that the response to be learned will occur. This system involves "strengthening of circuits" by repetitive use. Cholinergic influences on this system also have been suggested (Carlton, 1969). Thus, the alpha-adrenergic activating system may operate only when cholinergic neurons are not inhibiting learning processes.

Thut (1977) recently has suggested that serotonergic systems are involved in the impairment of CAR acquisition produced by L-dihydroxyphenylalanine (L-DOPA). He suggested that L-DOPA releases 5-HT, inhibits 5-HT synthesis by competing with 5-hydroxytryptophan (5-HTP) for L-aromatic-acid-decarboxylase, and produces increases in DA levels which result in competition for 5-HT binding sites. He hypothesized that the initial release of 5-HT after L-DOPA treat-
ment depresses CAR acquisition.

2. Cholinergic Systems

As with the catecholamine systems, the influence of the cholinergic system on learning is difficult to interpret. The literature on this subject also is extensive but inconsistent. The action of many cholinomimetic drugs (for example, anticholinesterases) are biphasic (Rosic, 1970; Karczmar et al., 1973; Karczmar, 1974, 1976). The effects of these drugs depend on the type of learning paradigm employed, the dosage and route of administration (Matthies et al., 1974).

In general, the anticholinesterases and nicotinic agonists facilitate the acquisition of a CAR, although Rosecrans and Domino (1974) have reported that physostigmine depressed both the acquisition and performance of a CAR.

The peripheral actions of drugs which are used to study cholinergic influences on behavior are considerable, and often mask facilitation or inhibition of conditioned avoidance acquisition. For example, Brimblecombe and Buxton (1971) using an automated shuttlebox apparatus, studied the effects of eight atropinic drugs on avoidance conditioning. They found that three of the drugs enhanced conditioned avoidance learning, while three others produced significant decreases in avoidance responding. All of the muscarinic blocking agents studied, however, increased spontaneous locomotor activity.

Muscarinic cholinergic blocking agents (atropine and Scopolamine, for example) have been shown to impair the performance of previously trained animals, suggesting a role
for the cholinergic system in memory or retention (Oliverio, 1967; Karczmar, 1973). However, disruption of performance can be due to peripheral side-effects and locomotor disabilities.

3. Serotonergic Systems

There is considerable evidence supporting a role for 5-HT in learning (Wooley, 1965; Essman, 1970; Stein et al., 1973. In general, electrolytic lesions of the 5-HT containing cell bodies in the mid brain deplete 5-HT level and facilitate the acquisition of a CAR (Lorens et al., 1971; Yunger and Harvey, 1976), while administration of drugs which increase 5-HT levels (tryptophan and 5-hydroxytryptophan) inhibit active avoidance conditioning (Engel and Modigh, 1974). But, as is the case with manipulation of catecholamine and cholinergic systems, the effect of depletion of 5-HT on avoidance conditioning depends upon both the method of depletion and the behavioral paradigm employed (Kohler and Lorens, 1977; Lorens, 1978).

Stein and Wise (1974) have proposed that 5-HT acts as a modulator in a punishment system, while NE acts to modulate a reward system. This view was based on the ability of these transmitter agents (when applied directly to the brain) to impair or facilitate avoidance behavior.

4. Summary

A case can be made for catecholaminergic, cholinergic and serotonergic involvement in learning processes. The effects
of manipulating the metabolism of these neurotransmitters on avoidance learning depend on 1) the method of manipulation (pharmacological treatment versus lesion method); 2) the site or route of drug administration; 3) the separation of learning from non-learning variables (for example, locomotor activity); and 4) the type of behavioral paradigm used.

B. EXPERIMENTAL DESIGN:

Effect of Neurotransmitter Modifying Drugs on the Acquisition of a CAR, Neurochemistry and Activity.

The acquisition of a CAR was studied in 8 groups of CF-1 mice using the "climbing screen" method (Chapter II, p. 7). The drugs, their mechanism of action, and dosage are presented in Table III. The groups (n = 10/group) received the following drugs based on previous pilot studies in our laboratory and dose response data which demonstrated those doses which provided appropriate behavioral and biochemical responses.

- Group 1) L-dihydroxyphenylalanine (L-DOPA)
- Group 2) L-5-hydroxytryptophan (L-5-HTP)
- Group 3) Alpha-methyl-para-tyrosine (α-MPT)
- Group 4) Para-chlorophenylalanine (p-CPA)
- Group 5) Physostigmine
- Group 6) Scopolamine hydrobromide
- Group 7) α-MPT + L-5-HTP + Physostigmine
- Group 8) Saline or gum tragacath (2mg/ml saline)

Eight additional groups (n=10/group) of naive mice were not tested in the conditioning paradigm, but received the same pharmacological treatments. They were sacrificed at
<table>
<thead>
<tr>
<th>DRUG</th>
<th>MECHANISM OF ACTION</th>
<th>ACTION ON TRANSMITTER LEVELS</th>
<th>DOSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Dopa</td>
<td>NE and DA precursor</td>
<td>Increase NE and DA</td>
<td>50 mg/kg (ip) 30 min prior to experiment</td>
</tr>
<tr>
<td>L-5-HTP</td>
<td>Immediate 5-HT precursor</td>
<td>Increase 5-HT</td>
<td>100 mg/kg (ip) 30 min prior to experiment</td>
</tr>
<tr>
<td>α-MPT</td>
<td>Tyrosine hydroxylase inhibitor</td>
<td>Decrease NE and DA</td>
<td>200 mg/kg (ip) 24 hr prior to experiment</td>
</tr>
<tr>
<td>p-CPA</td>
<td>Tryptophan and phenylalanine hydroxylase inhibitor</td>
<td>Decrease 5-HT</td>
<td>316 mg/kg (ip) 72 hr prior to experiment</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>Cholinesterase inhibitor</td>
<td>Increase ACh</td>
<td>.01 mg/kg (ip) 30 min prior to experiment</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>Muscarinic receptor blocker</td>
<td>Decrease ACh</td>
<td>.3 mg/kg (ip) 30 min prior to experiment</td>
</tr>
</tbody>
</table>

L-dihydroxyphenylalanine: L-Dopa; L-5-hydroxytryptophan: L-5-HTP; Alpha-methyl-para-tyrosine: α-MPT; Parachlorophenylalanine: p-CPA; norepinephrine: NE; 5-hydroxytryptamine: 5-HT; acetylcholine: ACh; dopamine: DA; milligram per kilogram: mg/kg; intraperitoneally: ip.
the same post-injection time as the conditioned animals, and their whole brain levels of NE, 5-HT and ACh determined.

Eight additional groups (n=10/group) of naive mice received the same pharmacological treatments and were placed in the Lehigh Valley Electronics (LVE) activity chambers (Chapter II, p. 12). The activity levels of the animals were determined at the same time post-injection as the animals which received CAR training.

C. RESULTS

The results of the effects of drugs on neurotransmitter concentration, avoidance conditioning and locomotor activity are summarized in Table IV. The data are presented in terms of percent change from control. In general, the data relative to the levels of NE, 5-HT and ACh agree with our previous results (Richardson et al., 1970; Richardson, 1971; Karczmar, et al., 1973), and the findings of others (Glisson et al., 1972). L-DOPA had the expected effect of increasing NE level (p 0.001), without affecting 5-HT or ACh level. 5-HTP increased 5-HT level (p 0.001), without affecting NE or ACh levels, whereas α-MPT decreased NE level (p 0.01) without an effect on 5-HT or ACh. Physostigmine resulted in an increased ACh level (p 0.001), with no effect on 5-HT or NE, while p-CPA decreased 5-HT level without effects on NE or ACh. Scopolamine failed to affect brain neurotransmitter concentrations. This result was contrary to our earlier studies (Karczmar et al., 1973) which showed a decrease in ACh after scopolamine administration. Although the decrease in ACh found in the present experiment was large (-18%) the data failed to reach statistical significance due to the large variance.
TABLE IV. Effect of drugs on whole brain neurotransmitters, avoidance conditioning and activity. A t-test revealed L-Dopa significantly (p<0.001) increased NE level, without affecting 5-HT or ACh level. 5-HTP only increased 5-HT level (p<0.001), whereas \( \alpha \)-MPT only decreased NE level (p<0.01). Physostig. resulted in an increased (p<0.001) ACh level, while p-CPA only decreased 5-HT level. Scopolamine failed to affect brain neurotransmitter concentrations. Only \( \alpha \)-MPT significantly (p<0.05) increased and scopolamine significantly (p<0.05) decreased the total number of conditioned avoidance responses (CAR). Both L-Dopa and scopolamine, furthermore, decreased locomotor activity, (p<0.01) and (p<0.05) respectively. NM: not measurable. See Table III for further details. Table on following page.
## TABLE IV

EFFECT OF DRUGS ON WHOLE BRAIN NEUROTRANSMITTERS, AVOIDANCE CONDITIONING AND ACTIVITY

(Percent change from control)

<table>
<thead>
<tr>
<th></th>
<th>NE</th>
<th>5-HT</th>
<th>ACh</th>
<th>#CAR</th>
<th>Locomotor Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Dopa</td>
<td>78***</td>
<td>-15</td>
<td>-2</td>
<td>-16</td>
<td>-26**</td>
</tr>
<tr>
<td>L-5-HTP</td>
<td>4</td>
<td>124***</td>
<td>-3</td>
<td>-4</td>
<td>-4</td>
</tr>
<tr>
<td>α-MPT</td>
<td>-61**</td>
<td>6</td>
<td>6</td>
<td>20*</td>
<td>+18</td>
</tr>
<tr>
<td>p-CPA</td>
<td>+3</td>
<td>-73**</td>
<td>+4</td>
<td>-3</td>
<td>+7</td>
</tr>
<tr>
<td>Physostigmine</td>
<td>-7</td>
<td>8</td>
<td>88***</td>
<td>16</td>
<td>+14</td>
</tr>
<tr>
<td>Scopolamine</td>
<td>+12</td>
<td>-6</td>
<td>-18</td>
<td>-32*</td>
<td>-20*</td>
</tr>
<tr>
<td>α-MPT + L-5-HTP</td>
<td>-48</td>
<td>104**</td>
<td>129**</td>
<td>NM</td>
<td>NM</td>
</tr>
</tbody>
</table>

*: p<0.05
**: p<0.01
***: p<0.001

Details on preceding page.
In general, the appearance of the animals following drug treatment was similar to control saline animals. Occasionally muscle tremors were seen after physostigmine administration, especially during avoidance testing. \( \alpha \)-MPT resulted in some sedation, but the p-CPA and 5-HTP treated animals showed no gross neurological or behavioral abnormalities. Animals given p-CPA and \( \alpha \)-MPT lost weight (8 gms + 2) from the time of injection to the beginning of the experiment. The combination of physostigmine, \( \alpha \)-MPT and 5-HTP was not fatal but toxic. This toxicity was manifested as extreme tremors, piloerection and, at times, convulsions. The appearance of these symptoms led to an abbreviation of the avoidance conditioning session; and the data were not analyzed for this group.

As seen in Table IV, only \( \alpha \)-MPT significantly (p<0.05) increased and scopolamine significantly (p<0.05) decreased the total number of conditioned avoidance responses (CAR). Both L-DOPA and scopolamine, furthermore, decreased locomotor activity, (p<0.01) and (p<0.05) respectively.

D. DISCUSSION

The effects of the combination of \( \alpha \)-MPT, L-5-HTP and physostigmine on neurotransmitter levels were particularly interesting since to our knowledge the neurochemical and/or behavioral consequences of this regimen have not been studied. This combination resulted in large increases in brain ACh and 5-HT levels, and a large decrease in NE content. We used this particular combination of drugs in order to test the hypothesis that a decrease in brain NE concentration
with an associated increase in 5-HT and ACh levels should facilitate the acquisition of a conditioned avoidance response. Unfortunately, this combination of drugs proved so toxic that their behavioral consequences could not be analyzed.

As indicated by the increase in the number of conditioned avoidance responses (CAR), only α-MPT treatment resulted in a significant facilitation in avoidance conditioning. As discussed earlier (Chapter III), the facilitation of avoidance conditioning might be due to factors other than those involved in the learning process. In this regard, the α-MPT treated group showed a significant increase in the number of CAR's emitted, but no significant change in activity level. In contrast, scopolamine, decreased both the number of CAR's emitted and activity level. L-DOPA, however, reduced activity level, but did not significantly affect the number of CAR's observed.

The results suggest that a decrease in central NE, and probably DA (increases in NE and DA turnover?) can lead to enhanced CAR acquisition without concomitant changes in activity level. Reductions in activity level may be associated with significant impairment in CAR acquisition (as seen following scopolamine administration), but this is not necessarily always the case, as witnessed by the effects of L-DOPA injection.

The results of these pharmacological manipulations do not favor our hypothesis that combined and simultaneous changes in central catecholaminergic, serotonergic and cholinergic neurotransmitter systems facilitate the "coping" response or conditioned avoidance learning, and
all drugs and doses employed are known to have major peripheral actions, other than their possible effects on locomotion, which may interfere with an animal's preparedness to adapt to a stressful situation. The results, thus, do not argue convincingly for/or against our hypothesis.

If stress and drug-induced changes in neurotransmitter levels are important in facilitating avoidance conditioning, then both genetically determined variations in endogenous transmitter levels (Chapter VII) and changes in transmitter concentration induced by stressors other than shock (Chapter VIII) should have an effect on avoidance conditioning.
CHAPTER VII
MOUSE STRAINS, NEUROCHEMISTRY AND LEARNING

A. INTRODUCTION

For some time our laboratory (Scudder et al., 1969; Richardson and Scudder, 1971; Karczmar et al., 1973) has engaged in research utilizing several genera and strains of mice to gather information concerning the role of central neurotransmitters in mediating behavior. The strains investigated exhibit large differences in terms of their central neurochemical levels and behavioral profiles (Mandel and Ebel, 1974; Meyers, 1967).

In previous work (Scudder et al., 1969) we demonstrated that correlations existed between particular behaviors (avoidance conditioning) and biochemistry (Brain ACh level) across several genera and strains of mice (Karczmar et al., 1973).

Bailey (1971) has developed a method of genetic analysis using recombinant (RI) strains, which has been useful in tracing gene-behavior-biochemical mechanisms. The RI strains were derived from a cross between two unrelated but highly inbred strains of Mus musculus (C57BL/6 and BALB/C). The offspring subsequently were inbred. A second group of mice was derived from a cross between C57BL/6 and BALB/C mice, subsequently backcrossed to C57BL/6 for several generations, resulting in different congenic lines. Subsequently, each of the congenic strains was tested against the RI strains
by means of skin grafts to determine which RI strain carried the BALB allele and which strain carried the C57 allele at a given histocompatibility locus.

By this technique it was determined that a single gene was responsible for exploratory behavior (Oliverio et al., 1973), active avoidance, and plasma serotonin level (Eleftheriou and Bailey, 1972). Oliverio and coworkers (1974) have concluded that plasma/brain 5-HT and avoidance learning are controlled by a single major gene effect, and that these two traits do not correlate at random, indicating the existence of a relationship between 5-HT and the modulation of some learning process. These workers indicated more research was needed to determine the relationship between learning, 5-HT and other brain amines.

Based on the above studies, a congenic line of mice (C57BL-Bailey) characterized by high avoidance, high plasma 5-HT and low activity was developed by the Jackson Laboratory (Bar Harbor, Maine) and made available for research.

We were interested in this particular strain for several reasons: 1) Since this strain was characterized by high plasma 5-HT level, comparison of its learning ability with that of a strain with a significantly lower 5-HT content could prove heuristic; 2) Since the strain purportedly was characterized by a low activity level, we might eliminate the possibility of high motor activity interfering with the acquisition of a conditioned avoidance response (CAR), such as was seen in our previous experiment (Chapter V); 3) Biochemical studies on this strain might reveal relation-
ships between brain neurotransmitter levels and learning capability.

B. EXPERIMENTAL DESIGN

Mouse Strain Differences in Neurochemistry, Acquisition of a CAR, and Activity Level.

1. Neurochemical Studies

Whole brain levels of NE, 5-HT, Ach and GABA were determined in two strains of mus musculus: CF-1 and C57BL6/Bailey (BY). A total of 30 mice were used for this experiment, 15 from each strain. Prior to sacrifice, the mice were housed 10 per cage as described in Chapter II (p.7).

2. Behavioral Studies

Locomotor activity (Photoactometers, Chapter II, p.12) and CAR acquisition (climbing screen, Chapter II, p.7) were determined in 10 mice from each strain. The animals were housed 10 per cage as described above (Chapter II).

C. RESULTS

1. NE, 5-HT, Ach and GABA Levels in Whole Brain.

A t-test for independent means revealed the C57Bl/BY strain exhibited a significantly (p<0.05) lower level of NE, and higher levels of 5-HT (p<0.05) and Ach (p<0.02) than the CF-1 strain. GABA levels did not differ significantly.

2. Avoidance Conditioning
ENDOGENOUS WHOLE BRAIN CONCENTRATIONS OF NE, 5-HT, ACH AND GABA IN TWO STRAINS OF MUS.

<table>
<thead>
<tr>
<th></th>
<th>CF-1</th>
<th>C57BL/BY</th>
<th>% Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NE (ng/g) SEM</td>
<td>286 ± 19</td>
<td>225 ± 24 *</td>
<td>- 21</td>
</tr>
<tr>
<td>5-HT (ng/g) SEM</td>
<td>843 ± 41</td>
<td>987 ± 31 *</td>
<td>+ 14</td>
</tr>
<tr>
<td>ACh (nM/g) SEM</td>
<td>21.24 ± 1.91</td>
<td>24.98 ± 1.86 **</td>
<td>+ 16</td>
</tr>
<tr>
<td>GABA (μg/g) SEM</td>
<td>118 ± 8.2</td>
<td>127 ± 7.3</td>
<td>+ 6</td>
</tr>
</tbody>
</table>

A t-test revealed the C57BL/BY strain exhibited a significantly (p<0.05) lower level of NE, and higher levels of 5-HT (p<0.05) and ACh (p<0.02), than the CF-1 strain. GABA levels did not differ significantly. See Table III for further details.
As can be seen in Fig. 13, the C57BL/BY strain evidenced a more rapid acquisition of the CAR. The C57BL/BY strain also made significantly more CAR responses than the CF-1 strain (a total of 50 CAR's was possible for each mouse). Importantly, there were no significant between-group differences in climbing times. In fact, C57BL/BY strain learned to avoid, its latency becoming less than 5 secs after 20 trials contrary to CF-1 strains.

3. Locomotor Activity

There was no significant between strain difference in total activity level, nor was there a difference in activity level during the first 10 min of the session. Both groups showed habituation to the chamber as reflected by decreased activity counts over time. The total activity counts exhibited the CF-1 was 4286 ± 286 and C57BL/BY was 4194 ± 197.

D. DISCUSSION

The results of these experiments support the hypothesis that mouse strains which evidence high brain levels of 5-HT and ACh, and a low NE content, acquire a CAR more rapidly than controls. The data also support the observation (Chapter VI) that activity level is not necessarily a predictor of avoidance conditioning ability, since the C57BL/ BY strain was not significantly different in locomotor activity than the CF-1 strain, yet acquired the avoidance task faster. In other words, activity level per se was not associated with more rapid acquisition of the CAR as was seen in the experiments of Williams (1971).

If the hypothesis is correct, and stress, or situations which mimic the neurochemical profile of stress (high brain
Figure 13. Mouse strain differences in avoidance conditioning. An analysis of variance indicated significant (p<0.003) strain and time, but not interaction effects. Significant (p<0.01, t-test) between group differences in response latency were seen during trials 21-25, 26-30, 31-35 and 40-45. The superiority of the C57BL/BY mice in the avoidance task also is indicated by their greater (p<0.05) total number of CAR's emitted (See inset.). For additional details see Fig.10.
levels of 5-HT and ACh, but low central NE content), facilitate the acquisition of a CAR, then other stressors which produce the same neurochemical profile also should facilitate CAR acquisition.

In order to further study the effects of stress on neurochemistry and learning, the effects of four stressors (other than foot shock) on avoidance conditioning and brain biochemistry were determined. These endeavors are elaborated in the next chapter.
CHAPTER VIII
NEUROCHEMICAL AND BEHAVIORAL
EFFECTS OF SENSORY DEPRIVATION AND
STRESSORS OTHER THAN FOOT SHOCK

A. INTRODUCTION

The literature concerning the neurochemical effects of the four experimental procedures (isolation, olfactory bulbectomy, glossopharyngealectomy, and enucleation) used in the following work is not extensive (cf. King and Cairncross, 1971; Pohorsely et al., 1971).

King and Cairncross (1973) found that olfactory bulb lesions did not appreciably affect a food rewarded bar press conditioned response in rats, but produced a deficit in avoidance conditioning which was related to a low activity level. Rats rendered anosmic by olfactory bulb lesions acquired the avoidance response more slowly, but were similar to sham operated controls in the total number of avoidance responses emitted. Marks et al. (1971) demonstrated that olfactory bulb lesions resulted in a superior performance by rats in a positively reinforced operant response. In that study, rats trained in avoidance tasks both active and passive, in contrast, were inferior to controls, and evidenced a lower activity level. Other studies have demonstrated that olfactory bulbectomy delays the acquisition of learned appetitive responses (Watson, 1970).

These same investigators used the technique of prior fear conditioning in one section of their study. This procedure involved the pairing of an unconditioned stimulus
(foot shock) with a conditional stimulus (opening a door or turning on a light). The animals then were exposed to the conditional stimulus alone.

There are some inconsistencies in the literature as to whether prior fear conditioning facilitates or depresses subsequent avoidance learning. The data on one-way avoidance (Baum, 1969; Slotnick, 1968), in general, suggests that a facilitatory effect predominates. De Toledo and Black (1967) reported that if shocks are paired with stimuli that signal danger, rats subsequently learn a one-way avoidance task faster than controls. Our earlier experiments (Chapter III-VI) with foot shock stress might be interpreted similarly. The observed facilitatory effect on avoidance conditioning may have in some way been due to pre-conditioning the mice to the fear of shock and the apparatus.

King and Cairncross (1971) also reported that bullectomy produced decreases in telencephalic NE content, but no changes in hypothalamic NE or plasma corticosterone levels, compared to sham operated controls.

Pohorecky et al. (1969) showed that after a unilateral section of the olfactory tract a reduction in telencephalic NE was seen ipsilateral to the lesion. There was no reduction in hypothalamic NE. King (1969) suggested that
of CA and 5-HT. Valzelli (1967) reported that such behaviors or stress did not affect levels of 5-HT. Welch and Welch (1968) found a decreased level in central NE subsequent to such behavioral adaptation. Lagerspetz (1968) found that brainstem NE was increased, in isolated mice. The whole brain turnover rates of both NE and 5-HT, moreover, have been reported to be reduced in isolated animals (Garattini et al., 1967; Welch and Welch, 1969; Brain, 1975). Valzelli and Garattini (1969) observed decreases in mes-and diencephalic 5-HIAA levels in isolated mice. Our laboratory has correlated decreases in brain CA and ACh content with aggressiveness in a variety of genera and strains of mice (Karczmar and Scudder, 1969). Cholinergic drugs, both agonists and antagonists exert dependable and consistent aggressive effects when applied to the limbic system and related structures (Decsi et al., 1969; Allikmets, 1974; Karczmar, et al., 1979). Also, besides eliciting various forms of aggression, cholinergic agonists facilitated muricidal behavior (McCarthy, 1969), while atropinics given intracerebrally or systemically blocked isolation-induced and muricidal aggression, as well as several forms of electrically-elicited aggression. Isolation did not affect choline acetylase (Consolo and Valzelli, 1970) or ACh turnover (Karczmar and Dun, 1978). Fighting among previously isolated animals has been reported to be without significant effect on the synthesis and turnover of brain CA when estimated as their accumulation after MAO inhibition,
and depletion after tyrosine hydroxylase inhibition (Welch and Welch, 1969). Modigh (1976) found that brain tyrosine hydroxylase activity accelerated rapidly when isolated animals were brought together and began to fight.

Based on the above studies, isolation would seem to be stressful to animals and may result in subsequent changes in brain neurochemistry and behavior. If isolation is indeed stressful, our hypothesis would predict a facilitation of conditioned avoidance response acquisition with increased brain levels of 5-HT and ACh, and a decreased central level of NE.
B. EXPERIMENTAL DESIGN

1. Glossopharyngealecctomy

Male CF-1 mice (n=72) were used in these experiments. Bilateral glossopharyngealecctomies (Chapter II, p.17) were performed on one group (n=24), while a sham operation was performed on another group (n=24). An additional 24 animals served as untreated controls. After the operation, the mice were housed 2 per cage for 4 weeks.

One half (n=12) of the mice from each group of operated, sham-operated, and untreated controls were tested for locomotor activity in the LVE activity chambers (Chapter II, p.12), and, subsequently, in the conditioned avoidance task (Chapter II, p.7).

The remaining half (n=12) of the mice in each group were sacrificed at a time corresponding to the completion of avoidance training in the above described animals, and their whole brain levels of NE and 5-HT determined.

2. Enucleation

Male CF-1 mice (n=180) were used in these experiments. Sixty mice were enucleated (Chapter II, p.18). A sham operation was performed on a second group (n=60). An additional 60 animals served as untreated controls. After the operation the mice were placed with their mothers for 6 weeks.
One half of the mice (n=30) from each group of operated, sham-operated and untreated controls were tested for locomotor activity in the LVE activity chambers (Chapter II, p. 12) and, subsequently, in the conditioned avoidance task.

The remaining half of the mice (n=30) in each group were sacrificed at a time corresponding to completion of avoidance training in the above described groups and their whole brain levels of NE and 5-HT determined.

3. Olfactory Bulbectomy

Male CF-1 mice (n=180) were used in these experiments. A bilateral olfactory bulbectomy (Chapter II, p. 18) was performed on one group (n=60), while a sham operation was performed on another group (n=60). Sixty additional animals served as untreated controls. After the operation the mice were housed 2 per cage for 4 weeks.

One half of the mice (n=30) from each group of lesioned, sham operated, and untreated controls were tested for locomotor activity in the LVE activity chambers (Chapter II, p. 12) and, subsequently, for their acquisition of a conditioned avoidance response in the "climbing screen" (Chapter II, p. 7).

The remaining half of the mice (n=30) from each group were sacrificed at a time corresponding to the avoidance training and their whole brain levels of NE and 5-HT determined.
4. Isolation

Male CF-1 mice (n=30) were isolated as described in Chapter II (p. 12). These were divided into 3 groups (n=10/group).

In the first group (n=10), isolation induced aggression was investigated (Chapter II, p. 12). Two mice were placed in the fighting chamber with a movable steel wall between them. After 15 sec the wall was removed and the 2 mice allowed to "fight", the aggressive mouse, and the latency in sec from the time of the removal of the dividing wall to the first appearance of either fierce wrestling or biting was recorded. Following completion of the experimental procedure, the two mice were returned to their individual cages, the fighting chamber was cleaned, and two different mice allowed to fight. In this way a "round-robin" of fights among the ten mice was achieved.

In the second group (n=10), locomotor activity was measured in the LVE activity chambers (Chapter II, p. 12). Subsequently these mice were tested, for their acquisition of a conditioned avoidance response in the "climbing screen" (Chapter II, p. 7).

Whole brain levels of NE and 5-HT were determined in a third group (n=10) of isolated mice at a time corresponding to the avoidance training in the second group.

Locomotor activity also was measured in 10 other aggregated male CF-1 mice which had received a total of 60
foot shocks immediately prior to being placed in the LVE activity chambers.

C. RESULTS

1. Biochemistry

None of the different stressors (glossopharyngeal resection, olfactory bulbectomy, enucleation and isolation) resulted in significant changes in whole brain NE or 5-HT concentrations (Table VI). The sham and operated animals were not different from unoperated controls.

2. Avoidance Conditioning

An analysis of variance failed to reveal a significant effect of any of the four stressors on CAR acquisition. Their rates of CAR acquisition were indistinguishable from that of the non stressed CF-1 strain shown in Fig. 13. The total number of CAR's for each of the four stressors is as follows:

<table>
<thead>
<tr>
<th>Stressor</th>
<th>Total CARs</th>
</tr>
</thead>
<tbody>
<tr>
<td>glossopharyngeal</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>olfactory bulbectomy</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>enucleation</td>
<td>28 ± 4</td>
</tr>
<tr>
<td>isolation</td>
<td>27 ± 3</td>
</tr>
</tbody>
</table>

3. Locomotor Activity

No significant differences in activity level for any of the experimental groups were found (Fig. 14). In the interest of clarity the sham operated animals are not included in the figure; they did not differ significantly from the untreated control animals.

4. Aggression

Isolation resulted in a significant (P < .001) increase in aggression. Seventy percent of the isolated animals fought. It was our intention to determine only if the animals were aggressive since aggressiveness might be an indicator of the
stressful nature of isolation in mice.


<table>
<thead>
<tr>
<th>Stressor</th>
<th>NE (ng/g) ± SEM</th>
<th>5-HT (ng/g) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glossopharyngealectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>276 ± 61</td>
<td>873 ± 50</td>
</tr>
<tr>
<td>Sham</td>
<td>230 ± 21</td>
<td>903 ± 39</td>
</tr>
<tr>
<td>Operated</td>
<td>247 ± 18</td>
<td>876 ± 36</td>
</tr>
<tr>
<td>Olfactory Bulbectomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>237 ± 51</td>
<td>906 ± 91</td>
</tr>
<tr>
<td>Sham</td>
<td>250 ± 19</td>
<td>840 ± 21</td>
</tr>
<tr>
<td>Operated</td>
<td>267 ± 25</td>
<td>843 ± 41</td>
</tr>
<tr>
<td>Enucleation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>233 ± 26</td>
<td>824 ± 91</td>
</tr>
<tr>
<td>Sham</td>
<td>290 ± 39</td>
<td>867 ± 12</td>
</tr>
<tr>
<td>Operated</td>
<td>241 ± 21</td>
<td>837 ± 66</td>
</tr>
<tr>
<td>Isolation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>268 ± 30</td>
<td>900 ± 24</td>
</tr>
<tr>
<td>Isolated</td>
<td>247 ± 36</td>
<td>871 ± 91</td>
</tr>
</tbody>
</table>
Figure 14. Failure of five different stressors to affect locomotor activity. An analysis of variance failed to reveal a significant treatment or interaction effect. The time effect, however, was highly significant \((p<0.001)\), indicating that the groups showed rapid habituation. Inset: Total activity counts during session following each stressor. A t-test revealed no significant differences in total activity between any of the groups. OB: olfactory bulbectomy; Gloss: glossopharyngeal-ectomy; Enuc: enucleation; Iso: social isolation; Shock: Foot shock stress (60 shocks, 1 per min).
D. DISCUSSION

The results of these experiments are quite surprising in light of the findings by King and Cairncross (1973). These authors found a decrease in NE level, and a decrease in the acquisition of a conditioned avoidance response after olfactory bulbectomy. In our experiments whole brain levels of NE were measured, whereas King and Cairncross (1973) measured hypothalamic and telencephalic levels of NE after olfactory bulb lesions. Thus, small changes may have been undetected in our whole brain analysis. Our experiments also failed to confirm the findings of King and Cairncross with regard to the slower acquisition of a conditioned avoidance response following olfactory bulbectomy. In short, none of the stressors employed in the present experiment affected either whole brain 5-HT or NE level, or CAR acquisition. Likewise, none of the stressors used produced a significant effect on activity level.

In our hands, isolation produced aggressive behavior but failed to affect whole brain monoamine concentrations, avoidance conditioning, or activity level.

Our hypothesis, therefore, was neither proven nor
disproven by this series of experiments, since no changes were seen in neurochemistry or behavior. The possibility exists that the stressors simply were not of sufficient strength to produce appreciable effects on whole brain neurotransmitter levels, avoidance acquisition or locomotor activity.
CHAPTER IX

SUMMARY, CONCLUSIONS AND SPECULATIONS

Mice were subjected to differing numbers of foot shocks. Whole brain NE level decreased, whereas 5-HT, ACh and GABA concentration increased as a result of 30 or more (but not 20 or less) foot shocks. The decrement in NE was evidenced only in the meso-diencephalon. The whole brain turnover rate of NE was increased, while the turnover rate of Ach was decreased after foot shock. Levels of 5-HIAA in whole brain after foot shock stress were not significantly altered, although they tended to increase.

Foot shock stress facilitated the acquisition of a conditioned avoidance response (CAR). However, of the drugs studied, only d -HPT, a tyrosine hydroxylase inhibitor, produced a facilitation in the acquisition of a CAR. This change in CAR acquisition was associated with a decrease in whole brain NE level.

Presumed reduction in ACh efficiency at muscarinic receptors following scopolamine administration reduced both motor activity level and the rate of CAR acquisition. The catecholamine precursor, L-DOPA, however, reduced activity level without affecting CAR acquisition. Drug induced changes in 5-HT level following either pCPA or 5-HTP, furthermore, did not affect either behavioral measure.
Mice which were shocked but allowed to escape in the avoidance conditioning "climbing screen" did not evidence any changes in the neurochemical measures studied. Furthermore, subsequent avoidance conditioning tended to "normalize" whole brain 5-HT, but not NE, levels, which had been changed due to prior foot shock stress.

A strain of Mus musculus (C57BL/BY), which exhibited lower endogenous levels of NE and higher levels of brain 5-HT and Ach compared to another strain of Mus musculus (CF-1), showed a superior performance in the avoidance acquisition task, but no significant difference in activity level.

Other stressors, including glossopharyngeal eclektomy, olfactory bulbectomy, enucleation, and social isolation, did not produce changes in brain 5-HT and NE concentrations, CAR acquisition or activity level.

The facilitatory effect of stress on avoidance learning, thus, probably was not due to increased locomotor activity. Rather, it is concluded that the shock-stress induced changes in the central concentrations and/or metabolism of NE, 5-HT and ACh (and possibly GABA) may cause the organism to assume a "readiness" posture, enabling it to react to novel situations more quickly and accurately. Once the task (trial and error) has been mastered (learning), the concentration and/or metabolism of the neurotransmitters (especially 5-HT) may return to "normal" levels. Fluctuations in the activity of these neurotransmitter systems, thus, may modulate the ability of an animal to adapt to stress and mediate the coping response.
These systems may modulate trial and error behavior causing certain behavioral sequences to be executed. The results of these experiments suggest, furthermore, that once an adaptive behavioral sequence has been established and consolidated, the neurotransmitter systems return to normal levels of activity.

The mechanism remains to be elucidated but these experiments suggest that during stress, catecholaminergic systems may be activated (increased turnover rate and decreased concentrations). During stress, cholinergic activity may be decreased (decreased ACh turnover and increased ACh level), thereby, facilitating consolidation.

The serotonergic system may be activated in stressful circumstances by a feedback mechanism, especially following activation of the pituitary-adrenal axis. During stress, plasma corticosterone levels are increased. A feedback action on 5-HT neurons is suggested by the results of intraventricular administration of 5-HT; an attenuation of the increase in plasma corticosterone caused by stress (Verme et al., 1973) was found.

A number of further experiments are suggested by these results. For example, the effect of "stress-relieving" drugs, such as chlordiazepoxide, on the neurochemical and behavioral profile during stress should be evaluated. It also would be interesting to test selective neurotransmitter receptor blocking agents and reuptake inhibitors on animals during stress. One sequel to the work reported in this thesis is
underway. Our laboratory (Scudder et al., 1976), and others (Kahn, 1975) have examined the alcohol preference of mice which exhibit a high endogenous brain level of serotonin and a low central norepinephrine content. The results suggest that those animals, especially Mus musculus C57BL/6 exhibit a higher preference for alcohol. It would be of interest to examine alcohol preference in foot shock stressed mice. Alcohol ingestion may be a "stress-relieving" agent in mice.

The results presented in this dissertation may serve as a guide to future studies concerning the role of select neurotransmitter systems in mediating distinct learning processes.


Aggression and the orienting reflex in several genera 
and strains of mice. Aggressologia 10:2-10.

Correlations between brain chemistry and ethanol 
preference in mice. In Drug Addiction. Vol. 4 "3rd 
International Conference on Drug Addiction". 
H. Lal and J. Singh, Eds. Stratton Intercontinental 
Medical Book Corporation, Pubs. 137-163.


Semiginovsky, B., Safanda, J., Sobotka, P., Jakoubek, B. 
and Pavlik, A. (1976). The cerebral GABA, Beta-alanine, 
Lysine and ethanolamine content and conversion of 
14C from U 14C D Glucose into their molecule during 
emotional stress in rats: Effect of Pyrithioxin and 

and Co.; Glenview, Ill.

Shalipina, V.G. (1973). Role of the brain adrenergic 
structures in non-specific responses of the organism 
to stimuli. Fiziol.Zh. SSSR. 58: 357-361.


Simmonds, M.A. (1971). Inhibition by atropine of the 

Slotnick, B. (1968), Effects of fear conditioning on the 
subsequent acquisition of an avoidance response. 

A study of acetylcholine levels in the whole brains 
of various genera and strains of mice and the effects 
of learning, isolation, and drugs on acetylcholine 


The dissertation submitted by Daniel L. Richardson has been read and approved by the following committee:

Dr. Alexander G. Karczmar
Chairman of the Committee
Chairman, Dept. of Pharmacology

Dr. Michael A. Collins
Department of Biochemistry

Dr. Silas N. Glisson
Depts. of Anesthesiology, Pharmacology

Dr. Alfred J. Kahn
Veterans Administration Hospital

Dr. Stanley A. Lorens
Department of Pharmacology

Dr. R. Alan North
Department of Pharmacology

The final copies have been examined by the director of the dissertation and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the dissertation is now given final approval by the Committee with reference to content and form.

The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[Signature]

[Signature]  Director's Signature