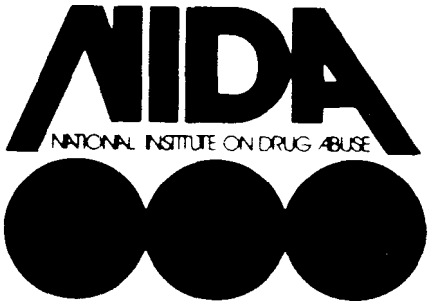
National Institute on Drug Abuse

Research

monograph series

3

AMINERGIC HYPOTHESES OF BEHAVIOR: REALITY OR CLICHÉ?



The NIDA Research Monograph series is prepared by the Research Division of the National Institute on Drug Abuse. Its primary objective is to provide critical reviews of research problem areas and techniques the content of state-of-the-art conferences, integrative research reviews and significant original research. Its dual publication emphasis is rapid and targeted dissemination to the scientific and professional community

EDITORIAL ADVISORY BOARD

NIDA RESEARCH MONOGRAPH series

Robert DuPont, M.D.	DIRECTOR, NIDA
William Pollin, M.D.	DIRECTOR, NIDA RESEARCH DIVISION
Robert C. Petersen, Ph.D.	EDITOR-IN-CHIEF
Eunice L. Corfman, M.A.	MANAGING EDITOR

Rockwall Building 11400 Rockville Pike Rockville, Maryland, 20852

aminergic hypotheses of behavior: reality or cliché?

edited by

bruce kenneth bernard, ph.d

school of pharmacy
university of connecticut
storrs, connecticut

november 1975

national institute on drug abuse, 11400 rockville pike, rockville, maryland 20852

Reproductions are

of Plates from the Seventh Book

of the ***De Humani*** **Corporis Fabrica**

of ***Andreas Vesalius*** *of Brussels,*

first issued at Basel, 1543.

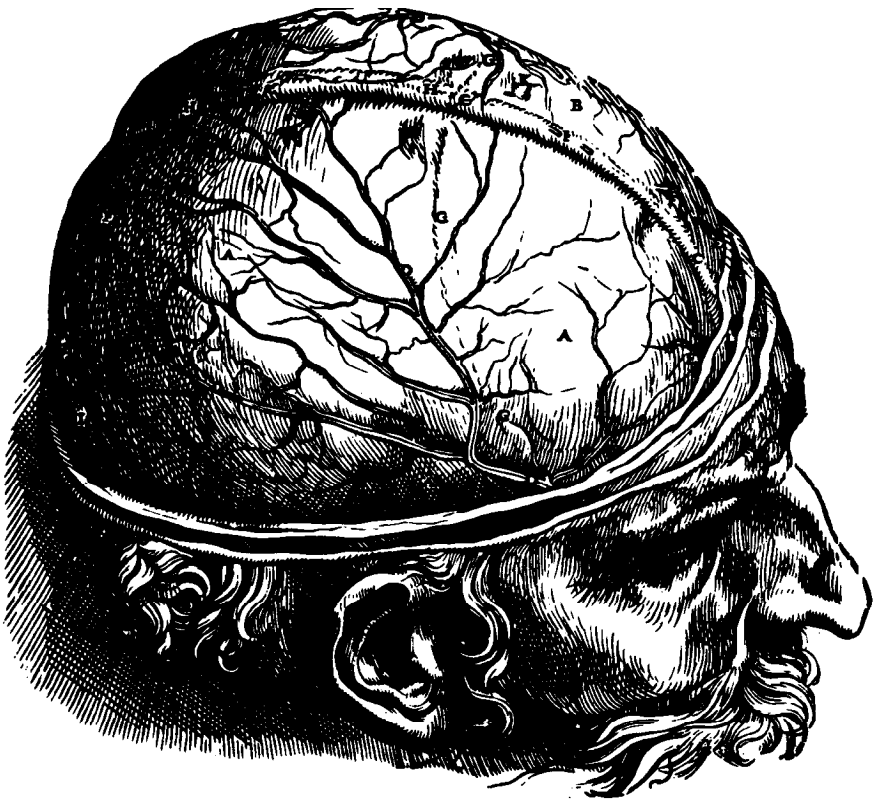
With permission of the

New York Academy of Medicine.

Library of Congress Cataloging in Publication Data

Main entry under title:
Aminergic hypotheses of behavior, reality or cliche?

(NIDA research monograph series; no.3)
1. Amines--Physiological effect. 2. Psychopharmacology. 3. Pharmacology, Experimental.
I. Bernard, Bruce Kenneth II. Series: National Institute on Drug Abuse. NIDA research monograph series; no. 3.
(DNLM: 1. Behavior--Drug Effects. 2. Amines--Metabolism. Wl RE232G no. 3 / QV129 A517)
QP981.A4A44 615'.78 75-33116


A
B
C
D
E
F
G

FOREWORD

The symposium from which the intriguing title of this volume is drawn poses a question which is difficult to assess in the aggregate, and for meaningful resolution might best be redirected to the symposium participants for their individual evaluation. The question ...reality or cliche?... suggests that one might simply sum a set of subquestions in an algebraic manner and arrive at a position with which persons of reason may be comfortable. Alas, while such a path might calm the spirit, it would only blur resolution of the question.

The dilemma presented to us may be illustrated by a story from the book "From Dream to Discovery (On Being a Scientist)" by Professor Hans Selye of the Institute of Experimental Medicine and Surgery, University of Montreal.

A blind beggar asked his friend, "Tell me, what do they mean by white? What is white like?"
"White is a color," he was told. "It is like the snow on the mountain."
"I see," the blind man said. "It is a cold and soft color."
"No, not always; paper is white, too."
"It is a thin and fragile color, then?"
"It need not be. Milk is also white."
"Is it fluid and nourishing?" the blind man asked, somewhat bewildered.
"Not necessarily," his friend explained. "All sorts of things are white: clouds, teeth, an old man's beard- your eyes are white, too, that is why you cannot see through them."
"Oh, never mind," the blind man sighed. "It is a cruel color. Perhaps it's just as well if I don't try to understand it."

Is the blind man plagued by his lack of vision, or is the inability of his friend to perceive the question and respond to its separate elements the root of the man's bewilderment and ultimate chagrin?

I submit that in attempting to provide explanations for complex behavioral patterns which are subserved and modulated by equally complex physiologic and biochemical processes we are assuming a risk that our response must necessarily be as faulty as that of the blind man's friend, The ultimate danger, I feel, is that we may becomes committed to the proposition that there must be an answer which is approachable with present tools and concepts. In order to provide explanations and to answer questions we fall prey to the measurement of amounts of things of one type (e.g., brain amines), which are accessible. What better wav to utilize such information than to correlate it with other, less well definable, observations (e.g., patterns of behavior)? A large part of science appears to be so directed.

Those who fall prey to this schema do so out of a noble motive which, unfortunately, only pushes the meaningful question further into the future. Our tendency is to frame global questions--out of our inability to discern the limits of the more discrete inquiries which we must make. Does anyone believe, for example, that we can at this juncture speak meaningfully to the point of the action of drugs on the brain? Can we describe experimentally accessible units of "brain" to which we direct such questions? The title of this volume and a reading of the contributions contained herein leave the impression that this group of scientists have not hesitated to contravene the conventional and the doctrinaire in their respective disciplines.

They have accepted and well responded to the challenge... "reality or cliche?

William F. Bousquet, Ph.D.
Department of Pharmacology and Toxicology
Purdue University, Lafayette, Indiana
currently: Director of Biological Research
Searle Laboratories, Chicago Illinois

CONTENTS

INTRODUCTION

The Title, "Aminergic Hypotheses of Behavior: Reality or Cliche?", describes the central question addressed by this monograph, namely, what is the status of our current views on the relation between the function of the brain monoamines and their effect on behavior? The idea of developing such a publication emerged in early 1974 following invitations to speak at the First world Congress of Biological Psychiatry (Buenos Aires, Argentina; September, 1974) and the Fifth Latin-American Congress of Pharmacology and Therapeutics (Lima, Peru; October 1974) on my research into the aminergic correlates of aggressive behavior. After discussions with researchers in related fields, a workshop entitled "The Functional Significance of Brain Monoaminergic Systems-Pharmacological and Biochemical Approaches" was organized and co-chaired by my good friend Dr. Sabit Gabay and myself at the Thirteenth Annual Meeting of the American College of Neuropsychopharmacology held in San Juan, Puerto Rico in December 10-13, 1974. This monograph contains several selected papers presented at that time and additional ones that were solicited for their appropriateness to the title topic.

The articles are arranged in an order such that the monograph progresses along a continuum of behaviors from those which are well-defined as causally related to the brain amines to others in which such a relationship is still highly speculative. Interspersed between the "area topic" articles, are papers narrower in scope and of a more technical nature, which have been chosen to explore in greater detail some point raised in the previous article. Articles of the first type are written by Drs. Hornykiewicz, Morgane and Stern, Bernard, and Sudilovsky; articles of the second type include those written by Drs. Thornburg and Moore, Shaskan and Becker, Cooper and Breeze, Ginsburg and Sze, Mandell and Knapp and Schildkraut, et al..

The casual relationship between the brain monoamines and parkisonism is probably one of the most well-defined findings in brain aminergic research to date. The first chapter, written by Dr. Oleh Hornykiewicz, deals with the relationship between brain dopomine and parkinsonism in addition to proposing an anatomical distinction between idiopathic and post-

encephalic parkinsonism. Furthermore, although pathologies of the dopaminergic neuronal system are central to the clinical entity, roles for norepinephrine in the akinesia and serotonin in the depression associated with the disorder are suggested. This paper concludes with a discussion of four pathophysiological and neuropharmacological factors which may impinge on the efficacy of l-dopa induced amelioration of clinical symptoms. Although conclusive human evidence for one of those proposed factors, supersensitivity of the dopwnine receptor, is difficult to obtain, data from animal studies such as found in the second paper by Drs. Thornburg and Moore and supportive of this hypothesized change in receptor responsiveness. Employing pharmacological manipulations which mimic the proposed result of parkinsonism, these author conclude that supersensitivity of dopamine receptors may play a role in these behavioral phenomena. Continuing the examination begun in the first paper of the relation between amines and affective disorders is the third paper written by Drs. E. Shaskan and R. Becker. In this study the authors attempted to differentiate schizophrenics from normals on the basis of high and low monoamine oxidase levels and although each MAO grouping occurred in normals, alcoholics and schizophrenics, the distribution of these groupings were similar across subjects.

Sleep is a second area in which research has sought to demonstrate a causal role for the brain monoamines. The fourth paper, written by Drs. P. Morgane and W. Stern, reviews the current experimental findings in relation to different sleep and waking states and concludes that serotonin and norepinephrine are involved in the "triggering" mechanisms required for these states of consciousness. The role of dopamine, as suggested in the first two articles, may be limited to behavioral arousal. However, as the paper clearly points out, the exact function of these pututive neurotransmitters and their interaction have yet to be elucidated. Several experimental behavioral paradigms, such as the self-stimulation experiments reported in the paper by Drs. B. Cooper and G. Breeze, provide additional evidence for the concept of behavioral activation induced by dopamine. After numerous pharmacological manipulations, these authors

conclude that although a role for norepinephrine in electrical self-stimulation cannot be definitively excluded, it is their belief that doparnine plays the major role in this behavior.

The papers thus far have for the most part emphasized single monoamineric systems in relation to a particular behavior. The concept of brain monoaminergic system balancing and integration for the control of a particular behavior is emphasized in the next paper which reviews a third topic area of amine research, aggression. In this article, I employed several hormonally, anatomically and pharmacologically distinct models of aggressive behavior and related each to divergent brain aminergic profiles. Thus, although correlations between aggressive behaviors and brain amines are evident, the functional significance of these findings is highly speculative. Furthermore, the possibility that different putative transmitter profiles may be important for different types of aggressive behavior increases the speculative nature of implications based on this data. However, the data do suggest the Cross-model importance of serotonin as a possible inhibitory transmitter. Support for this proposal is provided in the paper by Drs. B. Ginsburg and P. Sze in which the authors demonstrate a convariance in the genetic inheritances of audiogenic seizure susceptibility (and aggressive behavior) and tryptophan hydroxylase activity, the rate-limiting step in the serotonin biosynethetic pathy. This indoleamine may in fact be a critical factor involved in many types of animal affective behavior including that of humans. Paper eight, written by Drs. Mandell and Knapp describes the multiplicity of events by which brain serotonergic alterations can be induced. Furthermore, their data suggest that these alterations in serotonergic functioning are the mechanisms by which long term treatment with lithium acts prophylactically against both mania and depression. The authors seek to establish a neurobiological model of lithium's action, which although based upon a single amine, provides an explanation for both the increased and decreased Synthesis rates required for the observed behavioral effects.

The final area of brain amine research, touched upon in the previous paper, involves the relationship between brain monoaminergic systems and the behavioral effects of drugs. The first paper, written by Dr. A. Sudilovsky, describes the use of pharmacological agents for the production of a behavioral syndrome in animals which is comparable to the behavioral alterations seen in human amphetamine psychosis and certain forms of schizophrenia. These results can be interpreted as indicating a common underlying aminergic mechanism for these two syndromes. The last paper in this monograph, written by Schildkraut et al., examines explicitly the relation of the amines to drug behavior by examining the relation of heroin use to observed changes in urinary catecholamines and metabotites.

The above is, of course, simply an overview of the contents of this book and is provided as a guide to the reader in understanding the flow of the entire text. The implications and perhaps my own editorial biases can be found at the conclusion of the monograph.

It is my hope that this publication will be of value not only to researchers immediately concerned with the function of brain amines, but also to students of CNS pharmacology and others seeking a current overview of the field. The topics herein will provide the undergraduate with a foundation for understanding the pharmacological basis for the rational use of the therapeutic arsenal presently available to the health professions. For the advanced student, recent research, of which these articles are evidence, reveals the broad, heterogenous and fluid nature of presently held aminergic hypotheses of behavior and provides bracing indications that older, simpler suppositions are no longer adequate. This is always a salutory development and perhaps especially so for many researchers in this field of functional brain amine dynamics, who have felt that until recently their research had become inordinately parochial, each narrowly mining his own lode, and lacking sufficient concern for relation, perspective and generalizabitity. Nor is this concern recent. It was never more poignantly illustrated for me than when as a graduate student with several years of research accomplished I was faced with the following preliminary exam question:

"Many investigators over the past 10 or so years have been carrying out seemingly sophiscated biochemical experiments measuring such things as levels and the kinetics of synthesis and degradation of putative neurotransmitters peripherally and centrally. The supposed. objective of these studies is to define drug action, physiological mechanisms or behavioral events in terms of the suspected transmitter. I contend: (a) that this type of work has degenerated into a method-oriented, correlate-conscious cult from which little of value has emerged, (b) that the total approach is naive and fundamentally unsound and, (c) that the likelihood of this type of study yielding the above information is nil. Respond."

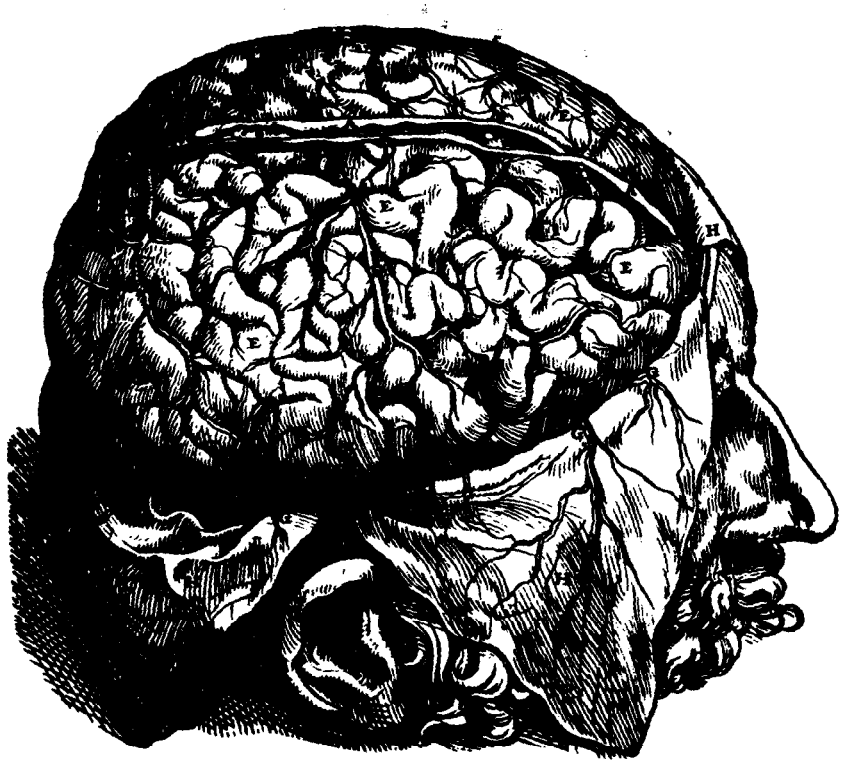
Such a sobering question, implicitly calling for critical evaluation of the meaning of one's work, is not easily dismissed. This monograph, with its coverage of widely divergent behaviors, provides some of the facts and comparisons that are prerequisite for that difficult and continuing evaluation.

Acknowledgements of debts owed are bound to be inadequate. Those left unspecified are not therefore unremembered or unthanked. But I wish especially to tender my gratitude to Dr. Peter Morgane for his support during the trying times of this monograph preparation, to Dr. Robert E. Willette of the National Institute on Drug Abuse, and to all fellow researchers who from time to time want to ask, "What is the meaning of what I do?"

A final thanks:

We are who we touch
and who touches us
betty & jack
gida & mike
carie lee & Cathy

Bruce K. Bernard



ABSTRACT

Hornykiewicz, O. *Brain Monoamines and Parkinsonism.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington. National Institute on Drug Abuse Research Monograph 3, 1975.

In Parkinson's disease there is a derangement of the metabolism of at least 3 major brain monoamines, namely, dopamine (DA), norepinaphrine (NE) and serotonin (5-HT). Of these alterations the severe deficiency of DA in the striatum is most characteristic, being (a) found in Parkinsonian syndromes of any etiology and (b) significantly correlated with the degree of cell loss in the substantia nigra, and the severity of the main symptoms. On the basis of neurochemical-clinical correlations Parkinson's disease may be subdivided into (a) an asymptomatic stage during which the striatal DA deficiency may reach a marked degree but can be canpensated by the remaining DA neurons, and (b) the stage of decompensation (i.e. clinically manifest disease) which ensues when the depetion of striatal DA reaches 70% or more. L-Dopa's main feature as a specific antiparkinson drug may be seen in its potential to revert the decompensated stage of the disease to the stage of functional compensation. This is in many cases possible because (a) the DA tumover in the remaining DA neurons is increased, providing for a high rate of formation (from L-dopa) and release of DA; (b) the "denervated" striatal receptors are supersensitive to DA; and (c) the newly-formed DA can be expected to reach a wide area of the striatum due to the high degree of divergence of the dopaminergic innervation. Compared with the striatal tal deficiency, the degree of NE and 5-HT decrease in the Parkinsonian brain is moderate. The decrease in NE may be due to the (moderate) cell loss in the locus coeruleus; at present no morphological basis for the lowering of brain 5-HT is known. The functional significance of the changes in brain NE may be an aggravation of akinesia. The decrease in brain 5-HT may be related to aspects of Parkinson's disease in turn related to affective behavior and mood.

KEY WORDS: Brain amines Dopamine Norepinephrine Serotonin Parkinson's disease

Department of Pharmacology and Department of Psychopharmacology, Clark Institute of Psychiatry, University of Toronto, Toronto Canada M5T lR8.

ABSTRACT

Thornburg, J.E. and Moore, K.E. *Supersensitivity to domminerаic agonists induced by haloperidol.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington. National Institute on Drug Abuse Research Monograph 3, 1975.

Haloperidol caused a significant reduction in the spontaneous locomotor activity of mice when added to their diet for 11 days. Upon removal of the drug from their diet these mice exhibited withdrawal hyperactivity for several days that was characterized by an increase in activity over control or pre-halopericol values. Similar results were obtained when mice were fed a diet containing pimozide. Withdrawal hyperactivity was not detected after 1 or 3 days of a haloperidol containing diet, but was maximal after 6 days of this diet. Dose-response curves of apomorphine-stimulated motor activity and rearing behavior were shifted to the left when determined in mice during the period of withdrawal hyperactivity. Dopaminergic agonists (apomorphine, piribedil, L-DOPA and d-amphetamine) induced gnawing at lower doses in mice removed from a chronic haloperidol-containing diet for 2 days than in mice maintained on a control diet. These results support the hypothesis that prolonged blockade of central dopaminergic receptors by neuroleptics causes subsequent behavioral effects that may be due to the development of enhanced receptor sensitivity.

KEY WORDS: Apomorphine Haloperidol Supersensitiity

Department of Pharmacology, Michigan State University, East Lansing, Michigan, 48824.

ABSTRACT

Shaskan, E.G. and R.E. Becker. *Blood platelet monoamine activity in anergic schizophrenics.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche? B.K. Bernard. ed., Washington, National Institute on Drug Abuse Research Monograph 3, 1975.

Blood platelet monoamine oxidase (MAO) activity was evaluated in twenty-four anergic, schizophrenic outpatients during a double-blind study comparing a chlorpromazine-imipramine combination to thio-thixene-placebo. Platelet MAO activity was determined on blood samples drawn after a two-week drug-free washout and once weekly over a four-week on-drug period. Schizophrenic patients could be classified according to their blood platelet MAO activity into either a low-MAO or a high-MAO group. In neither group of this population of schizophrenics did blood platelet MAO activity correlate with any of the primary or secondary symptoms of schizophrenia . Ten alcoholics and seven volunteer non-patients could similarly be divided into low- and high-MAO groups. Mean blood platelet MAO activity for these groups was not significantly different from the mean values of the low and high-MAO groups of the schizophrenics. These findings do not support published reports of low blood platelet activity as a genetic marker for schizophrenia. Discriminate function analysis of symptomatology ratings at baseline was used to characterize the low- and high-MAO schizophrenic patient groups. Individuals in the low-MAO group were distinguished by hyperactivity, anergia and sleep disturbance.

KEY WORDS: Chlorpromazine Human Imipramine Monoamine oxydase Platelets Schizophrenia Thiothixene

Department of Psychiatry, University of Connecticut School of Medicine, Farmington, Connecticut, 06032.

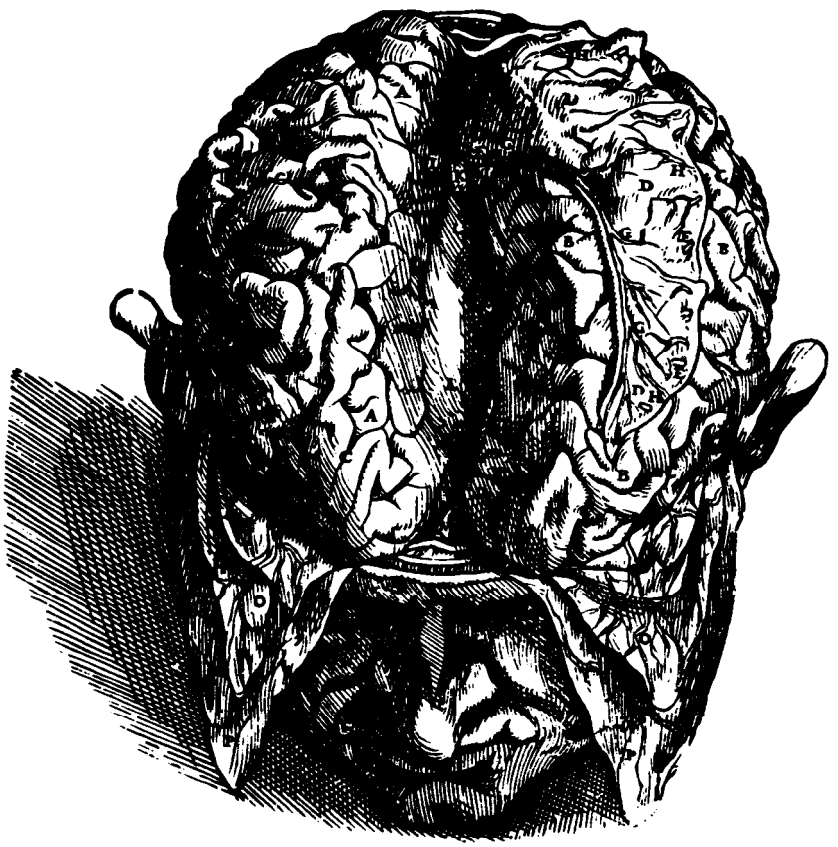
ABSTRACT

Morgame, P.J. and W.C. Stem. *The role of serotonin and norepinephrine in sleep-waking activity.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National Institute on Drug Abuse Research Monograph 3. 1975.

A critical review of the evidences relating the biogenic amines serotonin and norepinephrine to the states of slow-wave and rapid eye movement (REM) sleep is presented. Various alternative explanations for specific chemical regulation of the individual sleep states, including the phasic events of REM sleep, are evaluated within the overall framework of the monoamine theory of sleep. Several critical neuropsychopharmacological studies relating to metabolism of the amines in relation to sleep-waking behavior are presented. Models of the chemical neuronal circuitry involved in sleep-waking activity are derived and interactions between several brainstem nuclei, particularly the raphe complex and locus coeruleus, are discussed. Activity in these aminergic systems in relation to oscillations in the sleep-waking cycles is evaluated. In particular, the assessment of single cell activity in specific chemical systems in relations to chemical models of sleep is reviewed. Overall, it appears that the biogenic amines, especially serotonin and norepinephrine, play key roles in the generation and maintenance of the sleep states. These neurotransmitters participate in some manner in the "triggering" processes necessary for actuating each sleep Phase and in regulating the transitions from sleep to waking activity. The biogenic amines are, however, probably not "sleep factors" or direct inducers of the sleep states. Rather, they appear to be components of a multiplicity of interacting chemical circuitry in the brain whose activity maintains various chemical balances in different brain regions. Shifts in these balances appear to be involved in the triggering and maintenance of the various states comprising the vigilance continuum.

KEY WORDS: Biogenic amines EEG-sleep Norepinephrine Serotonin Sleep Pharmacology Sleep-waking

Laboratory of Neurophysiology and Psychopharmacology, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, 01545.



ABSTRACT

Cooper, Barrett R. and G.R. Breese. A *role for dopamine in the psychopharmacology of electrical self-stimulation.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National Institute on Drug Abuse Research Monograph 3, 1975

The psychopharmacology of electrical self-stimulation of the lateral hypothalamus was studied using 6-hydroxydopamine, alpha-methyltyrosine, U-14, 624, and d-amphetamine. Reduction of brain dopamine, but not norepinephrine, with 6-hydroxydopamine produced an acute depression of responding which eventually recovered to pretreatment levels. A low dose of alpha-methyltyrosine, which did not affect responding in control rats, significantly depressed responding in the rats with brain dopamine reduced. This treatment did not alter responding of rats with norepinephrine reduced by 6-hydroxydopamine. A dopamine-beta-hydroxylase inhibitor, U-14,624, depleted norepinephrine an additional 70% yet failed to alter self-stimulation in any of the groups. In other experiments, the 6-hydroxydopamine treatment which reduced brain dopamine was found to block the facilitation of self-stimulation produced by d-amphetamine. This facilitation of lateral hypothalamic self-stimulation was not influenced by treatments which reduced brain norepinephrine. An experiment suggesting that dopamine is of importance to locus coeruleus self-stimulation is also described. Implications of these data indicating a role for dopamine in self-stimulation responding are discussed in relation to the "catecholamine hypothesis of self-stimulation."

KEY WORDS: Alpha methyltyrosine d-amphetamine Self-stimulation Lateral hypothalamus 6-OH dopamine

Department of Psychiatry and pharmacology, Biological Sciences Research Center of The Child Development Institute, University of North Carolina School of Medicine, Chapel Hill, North Carolina, 27514.

ABSTRACT

Ginsburg, B.E. and P.Y. Sze. *Pharmacogenetic studies of the serotonergic system in association with convulsive seizures in mice.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard ed Washington, National Institute on Drug Abuse Research Monograph 3, 1975.

Trytophan hydroxylase (TPH) activity was determined in whole brain from male C57BL/10/Bg and DBA/1/Bg mice at 14 different ages between postnatal days 4 and 33. Brain TPH activity was higher at every age in C57BL/10/Bg than in DBA/1/Bg mice, the difference being 30-50% after day 20. The apparent Km of the enzyme for substrate was identical (1.4×10^{-3} M) in both strains. The reciprocal F_1's between DBA/1/Bg and C57BL/10/Bg strains were similar in TPH activity, being slightly lower than the predicted midparental value. At 30 days of age, C57BL/6/Bg males also had high TPH activity, indistinguishable from the C57BL/10/Bg strain. Audiogenic seizure susceptibility in these strains and their hybrid F_1's was inversely correlated with their brain TPH activities. Similarly, aggression scores in these genotypes were also inversely correlated with TPH activities. These results indicate that seizure susceptibility and aggression in mice may be related to the serotonergic activity in the brain. In the case of seizures, ethanol-induced susceptibility to audiogenic seizures in mice was enhanced by reserpine, and the effect of reserpine could be reversed by 5-HTP but not by DOPA. Furthermore, p-chlorophenylalanine also enhanced such susceptibility, whereas alpha-methyltyrosine had no effect. In the withdrawal audiogenic seizures in mice during chronic ethanol treatment, adrenalectomy blocked the ethanol-induced increase of brain TPH activity and also prevented the withdrawal seizures. Our results are consistent with the hypothesis that the serotonergic system is among the components regulating excitability in the brain.

KEY WORDS: Aggressive behavior Convulsive seizures Pharmacogenetic Tryptophan hydroxylase Serotonin

Behavioral Genetics Laboratory, Department of Biobehavioral Sciences, University of Connecticut, Storrs, Connecticut, 06268.

ABSTRACT

Mandell, A.J. and S. Knapp. A *model of the neurobiological mechanisms of action involved in lithiem prophylaxis of bipolar affective disorder.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National Institute on Drug Abuse Research Monograph 3, 1975.

The effects of chronic administration of lithium chloride on the serotonin synthesizing apparatus in rat brain suggest a theoretical model that could explain how chronic treatment with lithium is prophylactic against both poles of affect in manic-depressive disorder. After three to five days of lithium chloride administration the V_{max} of high affinity uptake of (^{14}C)tryptophan into striate synaptosomes increased to 140% of control values, and tryptophan-to-serotonin conversion activity increased to about the same degree. These events were followed by an apparently compensatory decrease in the V_{max} of midbrain activity
cell body and striate nerve ending tryptophan hydroxylase activity. After 21 days of drug administration (^{14}C)-tryptophan uptake remained above control levels, and soluble midbrain and solubilized striate synaptosomal enzyme activity remained below control levels, but synaptosomal conversion activity had returned to control levels. In vitro, drug concentrations from 10 to 53 nM did not affect the enzyme activity, but did enhance the uptake and conversion measures. Also, increasing tryptophan levels either by pre-incubation with L-tryptophan in vitro or by the administration of L-tryptophan (20 to 60 mg/kg) in vivo enhanced the uptake and conversion measures. The data suggest the possibility that lithium pushes two complementary adaptive mechanisms to their capacities, and the net result is restricted but balanced function of serotonergic transmission in the brain.

KEY WORDS: Adaptive capacity Conversion Lithium Manic depressive disorder Midbrain Serotonin Striatum Tryptophan Tryptophan hydroxylase Uptake

Department of Psychiatry, School of Medicine, University of California San Diego La Jolla California 92037

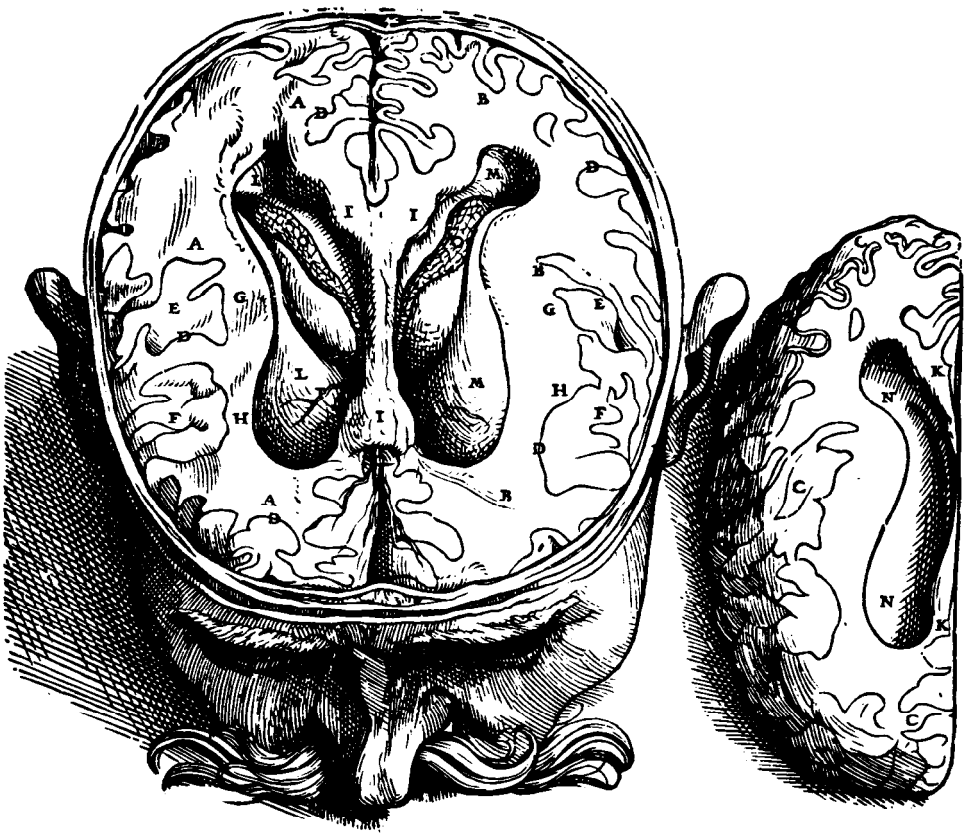
ABSTRACT

Bernard, B.K. *Aggression and the brain monoamines: what are the answers, but of more importance, what are the questions?* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National Institute on Drug Abuse Research Monograph 3, 1975.

Experiments involving three models of aggression (shock-induced fighting, ranacide and septal lesion-induced hyperirritability) are employed to demonstrate classically different sub-types of aggressive behavior. These categories are shown to be distinct entities when compared on the basis of hormonal dependency, central anatomical and peripheral autonomic involvement and inhibition or enhancement through pharmacological manipulations. Investigations into brain monoamine functioning (norepinephrine, dopamine and serotonin) demonstrate the heterogeneity of correlations which may exist between aggressive behaviors and brain amines. Data are analyzed on the basis of individual amine alterations and changes in monoaminergic neuronal balances. Thus, higher levels of shock-induced aggressive behavior is associated with higher NE/5-HT and DA/5-HT ratioes whereas similar alterations in these biochemical indices occur without observable changes in ranacide behavior. Septal lesion induced hyperirritability is correlated with precisely opposite aminergic changes, namely, decreases in NE/S-HT ratioes. These results demonstrate the necessity of precise aggressive model evaluation prior to attempts at biochemical mechanism elucidation.

KEY WORDS: Dopamine Norepinephrine Serotonin Septal lesion induced hyperirritability Shock-induced aggression

Division of Pharmacology and Toxicology, University of Connecticut, Storrs, Connecticut, 06268.


A
B
C
D
E
F
G
H
I
K
L
M
N

ABSTRACT

Sudilovsky, A. *Effects of disulfiram on the amphetamine-induced behavioral syndrome in the cat as a model of psychosis.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National lnstitute on Drug Abuse Research Monograph 3, 1975.

We have previously reported that, from a phenomenological standpoint, the behavioral manifestations of cats chronically intoxicated with amphetamine parallel the evolution of the paranoid psychosis induced by the drug in humans. However, certain manifestations in the cat, such as frozen postures, disjunctive behaviors and postures, cataleptic-like phenanena, obstinate progression, loss of righting reflex and pupillary changes, did not appear to be consistent with the phenomenology of the paranoid psychosis. Since treatment of schizophrenic patients with disulfiram, an inhibitor of norepinephrine synthesis that acts at the level of the enzyme dopamine beta-hydroxylase, thereby leading to increased dopamine concentrations, had been found to profoundly exaggerate psychotic symptanatology, the current experiments were undertaken to examine the amphetamine behavioral syndrome in the cat as it is modified by pretreatment with disulfiram.

Following such pretreatment, a faster development of certain end-stage components of the amphetamine syndrome was obtained. Thus, on the first day, development of a Reactive attitude and of more prominent behavioral disjunction occurred with the combined drug administration as compared with amphetamine alone. In contrast with the facilitation of these behaviors was the absence of dyskinesias and hyperreflexia on that day. Stereotyped behavior, loss of motor initiative and hyperkinetic activity were markedly enhanced and appeared with a shorter latency period on subsequent days of the intoxication cycle. Loss of righting reflex was an early manifestation *in* these animals.

During the later days, the particularly high level of compulsive activity was evident from the occurrence of an obstinate progression syndrome and the performance of stereotyped movements of the head in the presence of a crucifixion posture. In general, modification of the amphetamine effects on behavior was in a direction consistent with comparable features in experimental catatonia and the catatonic form of schizophrenia.

The need to integrate such phenomena in any amphetamine model of psychosis is stressed and analogies are drawn with similar features reported in animals treated with bulbocapnine or other psychotogenic compounds and with symptoms of human amphetamine psychosis and schizophrenia.

KEY WORDS: Amphetamine Disulfiram
Model Psychosis Stereotypy

E.R. Squibb and Sons, P.O. Box 4000, Princeton, New Jersey 08540.

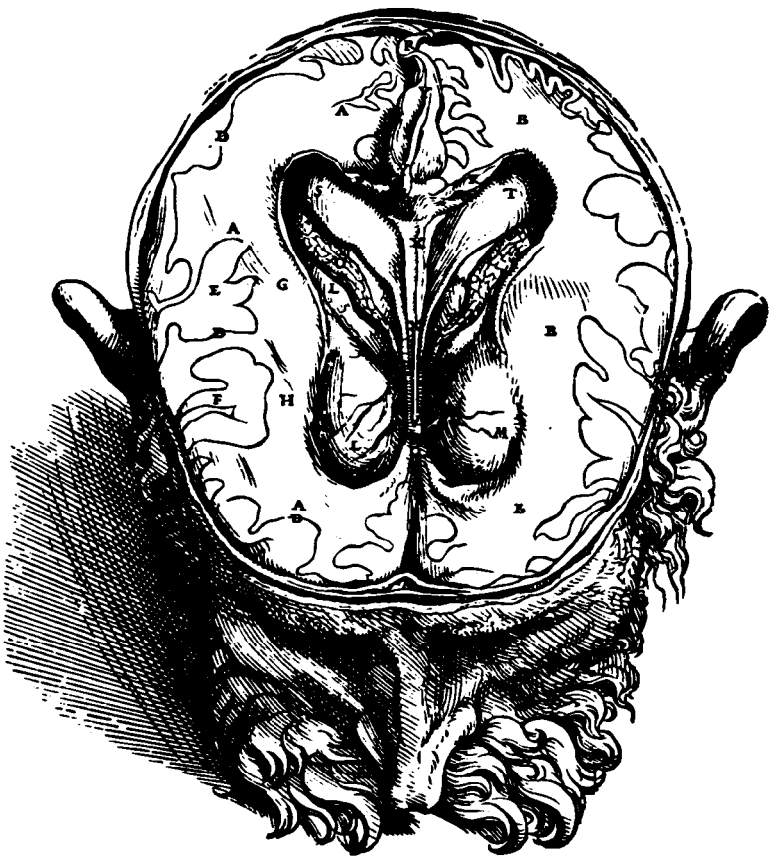
ABSTRACT

Schildkraut, J.J., R.E. Meyer, P.J. Crsulak, S.M. Mirin, M. Roffman, P.A. Platz, E. Grab, M.F. Randall, M. McDougle. *The effects of heroin on catecholamine metabolism in man.* IN: Aminergic Hypotheses of Behavior: Reality or Cliche?, B.K. Bernard, ed., Washington, National Institute on Drug Abuse Research Monograph 3, 1975.

In a study of the effects of heroin administration in nine human subjects, urinary catecholamines and metabolites were examined during an initial drug-free baseline period, a ten-day period of heroin administration and a subsequent period of methadone detoxification. All catecholamines and metabolites tended to be increased over baseline values on the first day of heroin administration. However, markedly different patterns of change emerged on subsequent days of heroin administration. Norepinephrine and normetanephrine remained increased throughout heroin administration. Epinephrine was increased during the early phase of heroin administration but returned to baseline values during the latter phase of heroin administration. After the increase on the first day of heroin administration, metanephrine decreased and substantial decrements below baseline values occurred during the latter phase of heroin administration. After increasing *on* the first day of heroin administration, 3-methoxy-4-hydroxy-mandelic acid (VMA) returned to approximately baseline values. During heroin administration, an *increase* in 3-methoxy-4-hydroxy-phenylglycol (MHPG) excretion was observed in a subgroup of four of the nine subjects studied. This is in contrast to the increase in normetanephrine excretion and the decrease in metanephrine excretion that was observed in the entire group of nine subjects. It is conceivable that persistance of, or development of, *tolerance might account* for the failure to observe an increase in MHPG excretion in all of the subjects. It appeared as if the increase in MHPG excretion began *on* the day prior to the administration of heroin in the subgroup of patients with increased MHPG excretionduring heroin administration, suggesting the possibility of an anticipatory *or* conditioned response, with the anticipation of heroin producing an increase in MHPG excretion.

KEY WORDS: Catecholamines Catecholamine metabolism
Norepinephrine 3-methoxy-4-hydroxyphenylglycol
Heroin Opiates

Neuropsychopharmacology laboratory, Massachusetts Mental Health Center, Boston, Massachusetts 02115.



BRAIN MONOAMINES AND PARKINSONISM

Oloh Hornykiowicz

INTRODUCTION

The advances made in the last 15 years in the field of the neurochemistry and neuropharmacology of Parkinson's disease provided the basis for the re-definition of this basal ganglia disorder as a "Striatal Dopamine Deficiency Syndrome" [23]. This new definition stresses the most prominent chemical abnormality so far detected in the brain of patients with Parkinson's disease. However, it is hardly necessary to point out that this term does not preclude the concomitant occurrence of other, less prominent, biochemical alterations which may be critically involved in the manifold clinical aspects of this disease, both of extrapyramidal and non-extrapyramidal origin. The following discussion deals with the changes in brain monoamines in Parkinson's disease. Other highly intriguing neurochemical alterations, such as the disturbance of the gamma-aminobutyric acid/glutamic acid decarboxylase system [cf. 24,28], is outside the scope of this presentation.

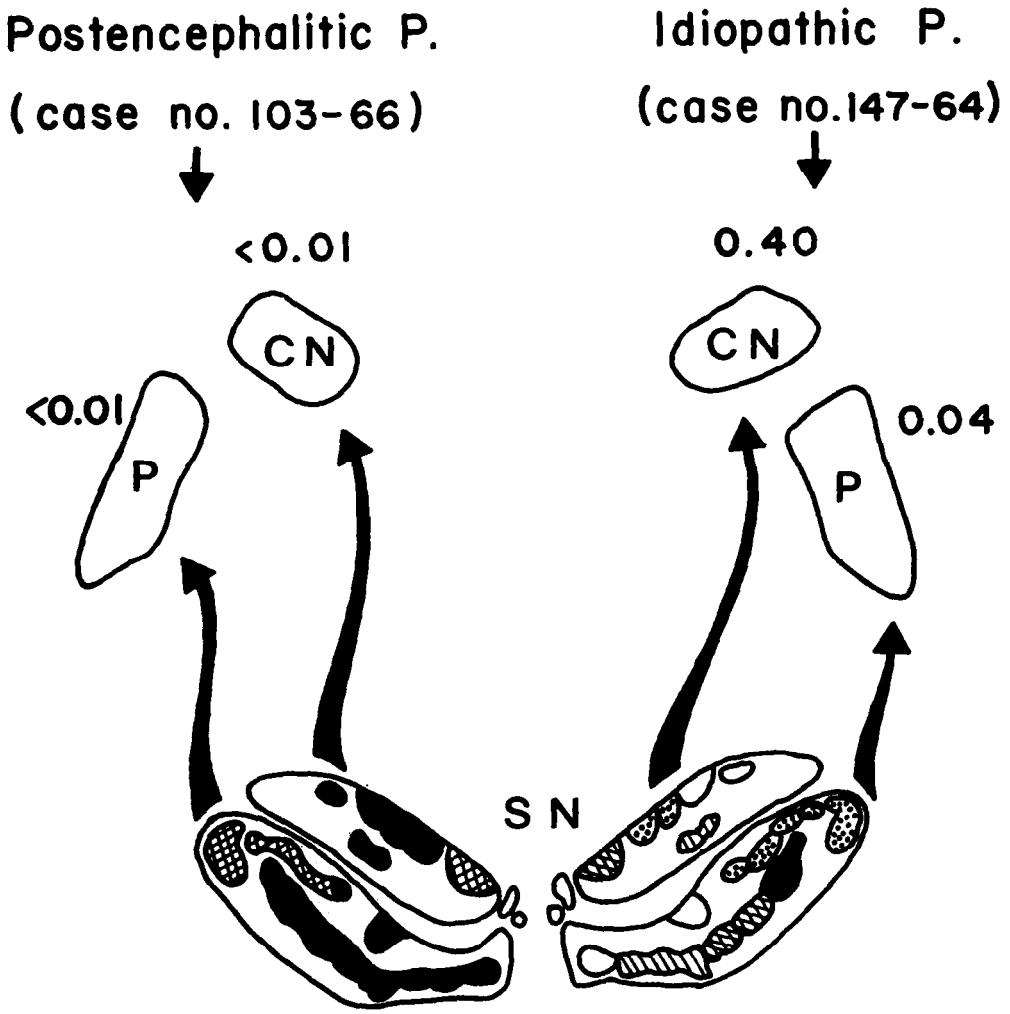
DOPAMINE

From a neurochemical point of view the most conspicuous feature of Parkinson's disease is the severe deficiency of dopamine (DA) and its metabolic endproduct homovanillic acid (HVA) in the basal ganglia, particularly the caudate nucleus, putamen (these nuclei constitute the striatum) and globus pallidus [9,16] as well as substantia nigra [20] (Table 1). These changes are typical of Parkinsons's disease regardless of etiology, being basically due to the degeneration of the melanin-containing neurons in the zona compacta of the substantia nigra [9]. It is now firmly established that these neurons give rise to the large nigro-striatal DA pathway [18]. Therefore, it is not surprising that in addition to the decrease in striatal DA and HVA levels, also the activity of the (intraneuronally localized) DA-synthesizing enzymes L-tyrosine hydroxylase (manuscript in preparation) and L-dopa decarboxylase [26,27] is decreased in the Parkinsonian striatum.

In view of the importance of the morphological state of the substantia nigra for the above chemical changes in Parkinson's disease, it is relevant that experiments in laboratory animals suggest the possibility of a somatotopic organization of the dopaminergic nigro-striatal projection. Based on these experiments it has been proposed that the rostral portion of the nucleus projects preferentially to the caudate nucleus, and the caudal portion to the putamen [6,12,30,31]. Results obtained in brains of patients with Parkinson's disease are in agreement with this conclusion. Thus in general, in idiopathic Parkinson's disease the decrease of DA and HVA is less severe in the caudate nucleus than in the putamen [9] (Figure 1). This most probably reflects the fact that, in general, in idiopathic Parkinson's disease the rostral portions of the substantia nigra are less severely degenerated than the caudal parts [19]. In contrast, in postencephalic Parkinsonism there is a diffuse and severe cell loss throughout the whole of the compact zone of the substantia nigra [19]; this correlates well with the finding that in this variety of

Parkinson's disease the DA deficiency is severe in degree and of similar magnitude in both the caudate nucleus and putamen [9] (Figure 1). The pathophysiological significance of this interregional difference in the two varieties of Parkinson's disease has not yet been fully explored.

FIGURE 1



Postencephalitic and idiopathic Parkineonirm (P): Relationship between the topography and degree of cell loss in the (rostra1 and caudal) compact zone of the substantia nigra (SN) and the dopamine (DA) levels in the caudate nucleus (CN) and putamen (P). Numbers related to CN and P are concentrations of DA in ug/g fresh tissue. The degree of SN lesion was assessed according to the following scale: = 0; = 1; = 2-3 = 4; = 5; for further details, cf. ref. (9).

In direct support of the concept that Parkinson's disease is a striatal DA deficiency syndrome observations can be cited showing that there. cause-effect relationship between the DA deficiency and the Parkinsonian symptomatology. In this respect three observations are particularly relevant: (a) in Hemiparkinsonism the neurochemical changes are more pronounced in the striatum contralateral to the affected side of the body [5] (Table 1); (b) the degree of' striatal DA (HVA) deficiency has been shown to be positively correlated with the severity of symptoms (notably akinesia and tremor) [9]; and (c) L-dopa, DA's immediate precursor substance, is highly efficacious in improving the main clinical features of this disease, especially akinesia [4,11,14].

TABLE 1

BRAIN DOPAMINE(DA) AND HOMOVANIILIC ACID (HVA) IN PARKINSON's DISEASE

Brain Region		Controls	Idiopathic	Postenceph-alitic	Arterioscler-otic-senile	Manganese Parkinson-iem	Hemiparkinson right	Hemiparkinson left
Caudate	- DA	2.64 ± 0.30 (28)	0.43 ± 0.09 (13)	0.04 ± 0.02 (6)	0.85 ± 0.26 (5)	0.35	1.25	0.59
nucleus	- HVA	3.23 ± 0.27 (8)	0.89 ± 0.12 (13)	0.29 ± 0.17 (6)	1.93 ± 0.49 (5)	ne	ne	ne
Putamen	- DA	3.44 ± 0.29 (28)	0.04 ± 0.01 (13)	0.02 ± 0.01 (6)	0.51 ± 0.28 (5)	0.04	0.93	0.13
	- HVA	4.29 ± 0.68 (8)	0.89 ± 0.12 (13)	0.38 ± 0.18 (6)	1.88 ± 0.40 (5)	ne	ne	ne
Subetantia	- DA	0.49 ± 0.09 (5)	0.07 ± 0.01* (10)	ne	ne	0.01	0.01	0.01
nigra	- **HVA**	1.79 ± 0.18 (5)	0.51 ± 0.15= (5)	ne	ne	ne	ne	ne
Globus	- DA	0.42 ± 0.08 (4)	0.10 (3)*	ne	ne	ne	ne	ne
pallidus	- HVA	2.12 ± 0.27 (8)	0.77 ± 0.12 (12)	0.24 ± 0.05 (6)	1.48 ± 0.42 (4)	ne	ne	ne

* Single case. ** In these cases no etiological differentiation was made. Values am expressed in ug/g wet tissue (where applicable as means ± S.E.M.); number of examined cases in parentheses. ne = not examined. Data taken from ref. (5) (9) (16) and (20).

It appears significant that the symptom of akinesia was positively correlated with the degree of loss of DA and HVA specifically in the caudate nucleus, but not in the putamen or globus pallidus [9] (Table 2). This is noteworthy in view of the fact that the caudate nucleus may receive its dopaminergic innervation predominantly from the rostral substantia nigra. Thus, it may be concluded that the dopaminergic pathway critically involved with striatal initiation of locomotor activity, to a large extent, has its cell bodies in the rostral portion of the compact zone of the substantia nigra and its terminals in the caudate nucleus.

TABLE 2

CLINICO-BIOCHEMICAL CORRELATION IN PARKINSON'S DISEASE: DEGREE OF AKINESIA VERSUS CONCENTRATIONS OF DOPAMINE (DA) AND HOMOVANILLIC ACID (HVA) IN CAUDATE NUCLEUS, PUTAMEN AND GLOBUS PALLIDUS

	Degree of akinesia	
	mild (group A)	marked (group B)
Caudate nucleus		
DA	0.58 ± 0.12 (13)	0.22 ± 0.08 (9)*
HVA	1.68 ± 0.25 (12)	0.59 ± 0.18 (7)**
Putamen		
DA	0.44 ± 0.21 (13)	0.05 ± 0.02 (9)
HVA	1.60 ± 0.39 (12)	0.83 ± 0.28 (7)
Globus pallidus		
HVA	1.30 ± 0.32 (10)	0.60 ± 0.17 (7)

* Significantly different from group A, $p<0.05$. **Significantly different from group A, $p<0.02$. values are expressed in µg/g (mean ± S.E.M.); number of examined cases in parentheses. Data taken from ref. (9).

As to the efficacy of L-dopa as an antiparkinson drug, a recent study revealed that in the Parkinsonian brain, L-dopa's metabolism basically proceeds along the same pathways as those established in the brain of normal laboratory animals. Thus, patients who received orally high amounts of L-dopa until death, had 9-15 fold higher levels of DA and HVA in the caudate nucleus and putamen than non-dopa treated patients [15,26]. In addition, low levels of dopa and higher levels of 3-0-methyl-dopa and HVA were measured throughout the brain. The biochemical basis for the metabolic transformations of L-dopa in the brain of patients with Parkinson's disease is indicated by the observations that (a) although markedly reduced, enough L-dopa decarboxylating activity remains in the Parkinsonian striatum to account for the formation of DA in these nuclei [27]; (b) the L-dopa decarboxylase activity in extrastriatal brain regions shows much smaller changes; and (c) both monoamine oxidase and catechol-O-methyl transferase have a widespread distribution in the brain, with no significant alterations in Parkinson's disease [26]. In this context a significant observation is that patients with a good response to L-dopa therapy achieved striatal DA levels several-fold higher than those of poor responders [26]. This evidence justifies the conclusion that in patients with Parkinson's disease, L-dopa acts as a DA replenishing drug.

Apart from the above observations directly supporting the role of the DA deficiency for the Parkinsonian symptomatology, there is a large body of (indirect) pharmacological evidence related to this question. In particular, it appears significant that drugs which either deplete DA or block DA release or its actions on the specific receptors, produce catalepsy in laboratory animals and in man a neurological syndrome often indistinguishable from Parkinson's disease [for ref. cf. 21,22].

The above observations describe in neurochemical terms the most prominent defect so far known in Parkinson's disease. However, in order to understand the functional, as well as pathophysiological and neuropharmacological implications of this neurochemical alteration, it appears profitable to ponder especially four specific aspects. These aspects can be regarded as being the direct consequence of the neurochemical changes typical of Parkinson's disease. They are as follows: (a) the state of reactivity of the DA receptors in the Parkinsonian striatum; (b) the state of activity of the remaining nigro-striatal DA neurons; (c) the high degree of divergence of the dopaminergic innervation of the striatum; and (d) compensatory brain mechanisms and the pathophysiology of Parkinson's disease.

State of reactivity of *DA receptors in the Parkinsonian striatum*

Clinico-pharmacological correlations have shown that there exists a positive correlation between the severity of akinesia and the patient's sensitivity to L-dopa [9]. It could be shown that patients with higher degrees of akinesia, and correspondingly higher degrees of striatal DA deficiency, responded more sensitively to a single i.v. test dose of L-dopa than patients with mild akinesia. Considered together with the observation that therapeutic doses of chronically administered L-dopa frequently produce in Parkinsonian patients, but not in control (=non-Parkinsonian) subjects,

dyskinetic, probably DA-mediated side-effects, it can be concluded that in Parkinson's disease the striatum becomes supersensitive to DA. This is not surprising as it has been known for a long time that denervation of a given tissue is often followed by the development of the phenomenon of "denervation supersensitivity" toward the respective neurotransmitter. Therefore the striatal receptors deprived of their dopaminergic innervation can be expected to develop this type of supersensitivity to DA.

Obviously, the development of such a "denervation supersensitivity" of the striatal receptors would greatly enhance any physiological effects of the DA formed from L-dopa. Thus, this phenomenon can be regarded as being part of compensatory changes triggered by the extensive loss of dopaminergic innervation of the Parkinsonian striatum. The physiological significance of this receptor supersensitivity for the efficacy of L-dopa in Parkinson's disease can be fully appreciated when considered together with the phenomena of compensatory overactivity of the remaining DA neurons and divergence of the dopaminergic innervation of the striatum (see below).

Level of activity in the remaining nigro-striatal dopamine neutrons

There *is* evidence to show that in Parkinson's disease the remaining nigro-striatal DA neurons are in a state of hyperactivity. Thus, the fact that the ratio of striatal "DA: HVA" is shifted in favour of the latter can be interpreted as an indication of an increased DA turnover (=increased rate of synthesis and release) and a corresponding decrease in the storage capacity for DA in the functionally intact neurons [21]. This interpretation would explain the additional observation that the levels of tyrosine hydroxylase activity in the Parkinsonian striatum were less severly decreased than those of L-dopa decarboxylase activity or DA (manuscript in preparation). This may indicate that there occurs in Parkinson's disease an induction of the rate-limiting enzyme activity in the striatum. These changes can be satisfactorily explained by assuming that in Parkinson's disease the loss of a large portion of the dopaminergic innervation of the striatum triggers a (receptor-mediated?) compensatory feedback activation of the remaining neurons. An analogous conclusion has been recently drawn from animal experiments with partial lesions of the nigro-striatal DA pathway [1]. Concerning L-dopa's mechanism of action the formation of large amounts of DA in, and increased rate of release from, the remaining overactive neurons may play an important role in the drug's therapeutic efficacy.

Divergence of nigro-stiatal DA neurons

It has been observed in the rat that the dopaminergic innervation that the striatum receives from the substantia nigra displays a high degree of divergence with one nigral DA fibre establishing hundreds of thousand synaptic contacts in the striatum [3]. The divergence of the dopaminergic innervation of the striatum most probably implies a high degree of innervation overlap and, therefore, can be taken to represent a considerable safety factor; that is to say, only loss of rather extensive portions of this innervation will deprive the striatal neurons of the critical minimum innervation beyond which functional compensation is impossible.

In conclusion, the combination of the factors quoted in the preceding paragraphs seems to ensure that (a) there will be high amounts of DA formed from L-dopa: (b) this DA will reach a wide enough area of the diseased basal ganglia; and (c) the newly formed DA will act upon. supersensitive receptors. As a consequence the dopaminergic control of striatal function will be restored.

Compensated and decompensated stages of Parkinson's disease

Although there is a significant positive correlation between the degree of disturbance of striatal DA metabolism and the symptoms of Parkinson's disease, even mild, clinically just detectable symptoms were associated with a disproportionately high degree of striatal DA deficiency [9]. This indicates that lesser degrees of DA deficiency can be functionally compensated by the striatum over longer periods of time. The biochemical basis for such a compensatory mechanism seems to be provided by the observation mentioned above, of a shifting in the

Parkinsonian striatum of the ratio "DA: HVA" in favour of HVA indicating an increase in DA turnover in the remaining functionally intact nigro-striatal DA neurons [21]. In the case of postencephalitic Parkinsonism, this compensatory mechanism may play a part in the phenomenon of the usually prolonged latency between the acute encephalitic infection and manifestation of the clinical symptomatology. Thus, clinically manifest Parkinsonism would appear to represent the second, decompensated stage of a process characterized by a steadily progressing disturbance of the striatal DA metabolism. From this, it seems reasonable to postulate that the main pharmacological feature of L-dopa as a specific, but mainly symptomatic, drug treatment of Parkinson's disease is its ability to revert the decompensated stage of the DA deficiency syndrome to that of functional re-compensation.

NOREPINEPHRINE

Apart from the severe cell loss in the substantia nigra, pathological changes (although less severe in degree) have been observed in other melanin-containing brain stem areas in Parkinson's disease. The most prominent of these areas is the locus coeruleus, where loss of melanin and formation of Lewy bodies have been described. It has been shown convincingly that the locus coeruleus is an eminently noradrenergic area, giving rise to norepinephrine (NE)-containing neurons innervating numerous structures at many levels of the CNS. Therefore, changes in NE levels in brain areas receiving inputs from the locus coeruleus could a priori be expected to occur in Parkinson's disease. This is in fact the case. Although no systematic studies have so far been performed, the available data indicate that the concentrations of NE in the Parkinsonian hypothalamus as well as the nuclei of the striatum, are distinctly subnormal [8,16] (Table 3). This observation is noteworthy because recently evidence has been accumulated showing that noradrenergic brain stem mechanisms may be critically involved in the control of locomotor activity [17]. Similarly, pharmacological evidence suggests that brain NE plays an important auxiliary role in the kinetic as well as the anti-akinesia effectiveness of dopaminergic drugs [25,29,32]. Thus, it may be postulated that the decrease of brain NE levels in Parkinson's disease plays an additional, but important, role in producing the symptom of akinesia, which is primarily related to striatal DA deficiency. Since there is little reason to doubt that NE is formed in the CNS of patients receiving L-dopa, it can be assumed that this NE will contribute to L-dopa's effectiveness as a specific anti-akinesia drug in Parkinson's disease. This possibility would help to explain why drugs acting predominantly (or exclusively) on dopaminergic mechanisms (such as apomorphine or piribedil) are considerably weaker than L-dopa as antiparkinson agents.

TABLE 3

BRAIN NOREPINEPHRINE IN PARKINSON'S DISEASE

Brain region	Norepinephrine	
	Controls	Parkinson's disease
Hyppothalamus	1.33 (2)	0.47 (5)
	(1.15 - 1.53)	(0.22 - 0.68)
Putamen	0.10 (3)	0.02 (6)
	(<0.01 - 0.15)	(<0.01 - 0.05)
Caudate nucleus	0.05 (2)	0.01 (6)
	(0.04 - 0.06)	(<0.01 - 0.02)
Glabus pallidus	0.02 (2)	0.01 (2)
	(0.01 - 0.02)	(<0.01 - 0.02)

Values are expresed in µ/g wet tissue; number of cases examined and range in parentheses. Date taken from ref. (8).

SEROTONIN

In Parkinson's disease, the levels of serotonin have been found to be subnormal in several brain areas [7] (Table 4). Most interesting presently appears the fact that there was a decrease in serotonin concentrations in such areas as the central grey of the midbrain and the hypothalamus. These brain stem areas have been shown to entertain numerous fibre connections with the structures constituting the forebrain limbic system. The latter

has been implicated in the control of mood and affect. This relationship assumes special significance if considered in the context that (a) the existence of a disturbance of brain serotonin metabolism has been postulated in mood disorders especially the depressive illness [13], and (b) mental depression is not infrequent in Parkinson's disease, its incidence in this disorder being as a matter of fact above the statistically expected average [2,10]. Therefore it is tempting to speculate that this may be related to the decreased levels of brain serotonin (and possibly NE) found in this disorder. This possibility represents a challenge to future research in the field of the neurobiology of psychotic disorders.

TABLE 4

BRAIN SEROTONIN IN PARKINSON'S DISEASE

Brain region	Serotonin	
	controls	Parkinson's disease
Central grey of midbrain	0.53 (6)	0.36 (6)
	(0.32 - 0.84)	(0.09 - 0.85)
Hypothalamus	0.29 (6)	0.12 (5)
	(0.14 - 0.51)	(0.06 - 0.21)
Thalamus	0.26 (4)	0.13 (4)
	(0.21 - 0.35)	(0.08 - 0.18)
Globus pallidus	0.23 (6)	0.13 (5)
	(0.19 - 0.29)	(0.07 - 0.22)
Putamen	0.32 (6)	0.14(5)
	(0.19 - 0.42)	(0.08 - 0.16)
Caudate nucleus	0.33 (6)	0.12 (5)
	(0.20 - 0.46)	(0.11 - 0.15)

Values are expresed in µ/g wet tissue; number of cases examined and range in parentheheses. Date taken from ref. (7)

REFERENCES

1. Agid, Y., F. Javoy and J. Glowinski. Hyperactivity of remaining dopaminergic neurones after partial destrauction of the nigro-striatal dopaminergic system in the rat. Nature New Biology 245, 150-151, 1973.

2. Ajuriaguerra, J.de, J. Constantinidis, J.J. Eisenring, G. Yanniotis and R, Tissot. Behaviour disorders and L-dopa therapy in Parkinson's disease. In: Parkinson's Disease, vol. 1, edited by J. Siegfried. Bern: Huber, 1972, PP. 202-217.

3. Andén N.E., K. Fuxe, B. Hamberger and T. Hökfelt. A quantitative study of the nigro-neostriatal dopamine neuron system in the rat. Acta physiol. scand. 67, 306-312, 1966.

4. Barbeau, A., T.L. Sourkes and G. Murphy. Les catecholamines dans la maladie de Parkinson. In: Monoamines Et Systems Nerveux Central, edited by J. De Ajuriaguerra, Georg, Geneva and Masson, Paris, pp. 247-262, 1962.

5. Barolin, G.S., H. Bernheimer and O. Hornykiewicz. Seitenverschiedenes Verhalten des Dopamin (3-Hydroxytyramin) in Gehirn eines Falles von Hemiparkinson. Schweiz. Arch. Neurol. Psychiat. 94: 241-248, 1964.

6. Bedard, P., L. Larochelle, A. Parent and L.J. Poirier. The nigrostriatal pathway: A correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey. Exp. Neurol. 25: 365-377, 1969.

7. Bernheimer, H., W. Birkmayer and O. Hornykiewicz. Verteilung des 5-Hydroxytryptamin (Serotonin) im Gehirn des Menschen und sein Verhalten bei Patienten mit Parkinson-Syndrom. Klin. Wschr. 39: 1056, 1961.

8. Bernheimer, H., W. Birkmayer and O. Hornykiewicz. Zur Biochemie des Parkinsonsyndroms des Menschen. Klin. Wschr. 41: 564-569, 1963.

9. Bernheimer, H., W. Birkmayer, O. Hornykiewicz, K. Jellinger and F. Seitelberger. Brain dopamine and the syndromes of Parkinson and Huntington. J. Neurol. Sci. 20, 415-455, 1973.

10. Birkmayer, W. Biochemistry of depression in Parkinson's disease. In: L-Dopa and Parkinsonism, edited by A. Barbeau and F.H. McDowell, Philadelphia: Davis, 1970, pp. 341-342.

11. Birkmayer, W. and O. Hornykiewicz. Der L-Dioxyphenylalanin (=L-DOPA) Effekt bei der Parkinson-Akinese. Wien. Klin. Wschr. 73: 787, 1961.

12. Carpenter, M.B. and P. Peter. Nigrostriatal and nigrothalamic fibers in the Rhesus monkey. J. comp. Neurol. 144: 93-177, 1972.

13. Coppen, A. Indoleamines and affective disorders. J. Psychiat. Res. 9: 163-171, 1972.

14. Cotzias, G.C., M.H. Van Woert and L.M. Schiffer. Aromatic amino acids and modification of parkinsonism. New Eng. J. Med. 276: 374, 1967.

15. Davidson, L., K. Lloyd, J. Dankova and O. Hornykiewicz. L-dopa treatment in Parkinson's disease: effect on dopamine and related substances in discrete brain regions, Experientia 27: 1048-1049, 1971.

16. Ehringer, H. and O. Hornykiewicz. Verteilung von Noradrenalin und Dopamin (3-Hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankungen des extrapyramidalen Systems. Klin. Wschr. 38: 1236-1239, 1960.

17. Forssberg, H. and S. Grillner. The Locomotion of the acute spinal cat injected with Clonidine i.v. Brin Research 50: 184-186, 1973.

18. Fuxe, K., T. Hökfelt and U. Ungerstedt. Morphological and functional aspects of central monoamine neurons. In: Internat. Rev. Neurobiology. Vol. 13, edited by C.C. Pfeiffer and J.R. Smythies, New York, London: Academic Press, 1970, PP. 93-126.

19. Hassler, R. Zur Pathologie der Paralysis agitans und des postenzephalitischen Parkinsonismus. J. Psychol Neurol. (Lpz.) 48: 387-476,

20. Hornykiewicz, O. Die topische Lokalisation und das Verhalten von Noradrenalin und Dopamin (3-Hydroxytyramin) in der Substantia nigra des normalen und Parkinsonkranken Menschen. Wien. Klin. Wschr. 73: 309-312, 1963.

21. Hornykiewicz, O. Dopamine (3-hydroxytyramine) and brain function. Pharmacol. Rev. 18: 925-964, 1966.

22. Hornykiewicz, O. Dopamine and its physiological significance in brain function. In: The Structure And Function of Nervous Tissue, vol. VI, edited by G.H. Bourne. Academic Press, Ind. New York and London, 1972, pp. 367-415.

23. Hornykiewicz, O. Dopamine and extrapyramidal function and dysfunction. In: Neurotransmitters, Res. Publ. A.R.N.M.D. vol. 50, edited by I.J. Kopin. Baltimore: Waverly, 1972, pp. 390-412.

24. Hornykiewicz, O. Metabolism of dopamine and L-dopa in human brain In: Frontiers in catecholamine researach, edited by E. Usdin and S.H. Snyder. New York: Pergamon, 1973, PP. 1101-1107.

25. Kuschinsky, K. and O. Hornykiewicz. Effects of morphine on striatal dopamine metabolism: Possible mechanism of its opposite effect on locomotor activity in rats and mice. Europ. J. Pharmacol. 26: 41-50, 1974.

26. Lloyd, K.G., L. Davidson and O. Hornykiewicz. Metabolism of levodopa in the human brain. In: Advances in Neurology vol. 3, edited by D.B. Calne, New York: Raven Press, 1973, pp. 173-188.

27. Lloyd, K. and O. Hornykiewicz. Parkinson's disease: Activity of L-dopa decarboxylase in discrete brain regions. Science 170: 1212-1213, 1970.

28. Lloyd, K.G. and O. Hornykiewicz. L-Glutamic acid decarboxylase in Parkinson's disease: effect of L-dopa therapy. Nature 243: 521-523, 1973.

29. Maj, J., H. Grabowska and E. Mogilnicka. The effect of L-dopa on brain catecholamines and motility in rats. Psychopharmacologia 22, 162-171, 197.

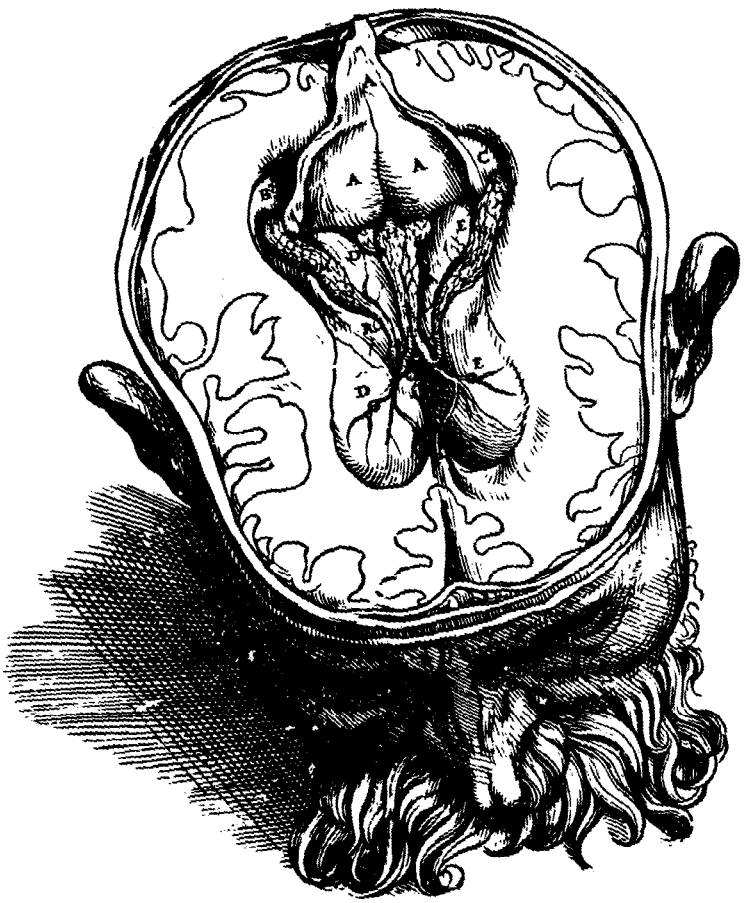
30. Moore, R.Y., R.K. Bhatnagar and A. Heller, Anatomical and chemical studies of nigro-neostriatal projection in the cat. Brain Res. 30: 119-135, 1971.

31. Riddell, D. and J.C. Szerb. The release in vivo of dopamine synthesized from labelled precursors in the caudate nucleus of the cat. J. Neurochem. 18: 989-1006, 1971.

32. Strömberg, U. and T.H. Svensson. L-Dopa induced effect on motor activity in mice after inhibition of dopamine-beta-hydroxylase. Psychopharmacologia 19: 53-60, 1971.

AUTHOR

Oleh Hornykiewicz, M.D.: Department of Pharmacology and Department of Psychopharmacology, Clarke Institute of Psychiatry, University of Toronto, Toronto, Canada, M5T 1R8.


A
A
D
D
E

SUPERSENSITIVITY TO DOPAMINERGIC AGONISTS INDUCED BY HALOPERIDOL

J.E. Thornburg, K.E. Moore.

INTRODUCTION

Prolonged disruption of nerve impulse transmission at central dopaminergic synapses with 6-hydroxydopamine [16,20,21,22] or alpha-methyltyrosine [2,6] leads to enhanced responses to direct and indirect acting dopaminergic agonists. Following chronic blockade of dopaminergic receptors with neuroleptics, the doses of dopaminergic agonists required to produce stereotyped behavior are reduced [6,8,12,17,18,23]. In each case, the enhanced responses to dompamine agonists may be due to supersensitivity of dopaminergic receptors.

The present study demonstrates that, following prolonged administration of haloperidol to mice, evidence of supersensitivity of dopaminergic receptors is manifested as increased spontaneous locomotor activity, increased apomorphine-stimulated motor activity and increased sensitivity to dopaminergic agonist-induced gnawing behavior.

METHODS

Experiments were conducted in male Swiss-Webster mice (Spartan Research Animals, Haslett, Michigan), with initial body weights of 20-25 g. These mice were provided with water and a diet of 20 g ground Wayne Lab Blox/4 mice/24 hrs (control diet) or the same diet containing 0.005% haloperidol or 0.01% pimozide.

Locomotor activity of groups of 4 mice was measured using Electronic Motility Meters 40Fc (Motron Products, Stockholm, Sweden) which have 40 infrared-sensitive photocells spaced 4 cm apart in the base and a light source overhead. These units were placed in a sound-proof, fan-ventilated chamber (5'x5'x3'). For testing, the animals were placed in a plexiglass cage (18"x 10" and 5") which just covers the photocell-sensitive area of the Meters. In experiments in which nocturnal activity was measured, the mice were maintained continuously in the test environment. Activity was recorded and food presented from 1700 to 0800 hrs.

Hearing activity was determined using a Vertical Movement Detector 5Fc (VMD, Motron Products) attached to the Motility Meter. The VMD consists of 5 photocells at one end of the cage, each directly opposite a light source. The photocells were positioned 2" above the base, so that a standing or rearing movement was necessary to activate the photocell.

The ability of various dopamine agonists and central stimulants to induce gnawing or chewing behavior in mice was determined as described by Pedersen and Christensen [14]. Following an injection of drug, pairs of mice were placed in a 4-walled chamber (10-1/2"x5"x12") which rested on a sheet of corrugated cardboard.

After one hour in the chamber, mice were removed and the presence or absence of gnawing on the cardboard was noted. The characteristic drug-induced gnawing must be distinguished from the qualitatively different ripping or shredding of the corrugated paper by saline-treated control mice.

All motor activity data were analyzed using Student's 't' test and gnawing behavior data were analyzed using a Chi square contingency test [9]. The level of significance was chosen as $P<0.01$.

DRUGS

Haloperidol and pimozide, obtained from Dr. John Kleis, McNeil Labs, Fort Washington, Pa., were incorporated into the diet of ground food. Apomorphine HC1, purchased from Eli Lilly and Co., Indianapolis, Indiana, was dissolved in 0.1% sodium metabisulfite. Piribedil (ET495, Trivastal), obtained from Dr. J. Pearl Sterling-Winthrop Research Laboratories, Hennselaer, L-alpha-methyldopahydrazine (MK486), obtained from Dr. C.A. Stone, Merck liesearch Laboratories, West Point Pa., and L-DOPA, Nutritional Biochemicals, Cleveland, Ohio, were suspended in **1%** methylcellulose. Benztropine mesylate, obtained from Dr. C.A. Stone, Merck Sharpe and Dohme Research Laboratories, West Point, Pa., d-amphetamine Sulfate, obtained from Dr. B.A. Kimes, Smith Kline and French Laboratories, Philadelphia, Pa., and methylphenidate HCl, obtained from CIBA-Geigy Corporation, Ardsley, N.Y., were dissolved in saline.

RESULTS

Spontaneous Motor Activity During and *After Chronic Diets of Haloperidol and Pimozide*

Nocturnal activity of groups of 4 mice, measured in their home environment, was reduced by 60 to 75% during an 11 day period in which 0.005% haloperidol was added to their diet. Upon reinstitution of a control diet, activity increased to 150 to 175% of pre-haloperidol activity (Fig. 1). This increased activity was maintained for 5 days after withdrawal of the haloperidol diet. In a similar experiment in which mice were fed a diet containing 0.01% pimozide for 10 days nocturnal activity was reduced by 40 to 70% (Fig. 2). During the first 3

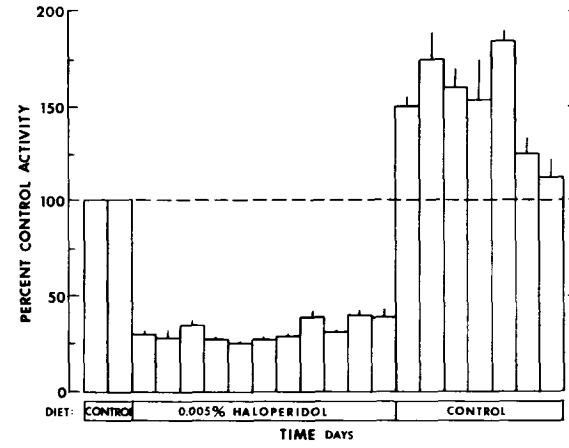


Figure 1

Figure 1. Spontaneous locomotor activity of mice during and after a chronic diet containing haloperidol. Activity of groups of 4 mice was recorded in the home environment during the dark period (1700-0800 hr); a control diet or a diet containing 0.005% haloperidol was presented during these hours. Total nocturnal activity (bars and vertical lines represent mean ± 1 S.E.) is expressed as percent of control of the mean activity for the two nights preceding the addition of haloperidol to the diet. Activity was significantly increased on the first 5 days following the haloperidol diet ($P<0.01$).

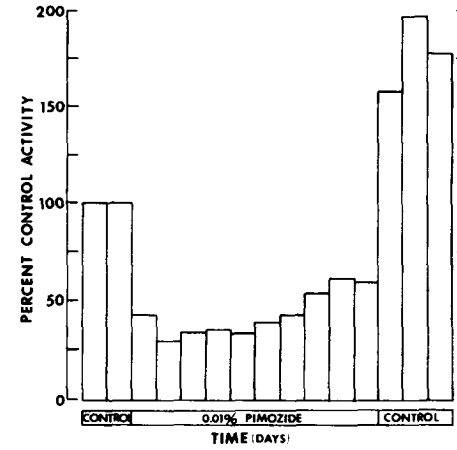


Figure 2

Figure 2. Spontaneous locomotor activity of mice during and after a chronic diet containing pimoaide. The experimental protocol is described in the legend to Fig. 1 except that mice were fed a diet containing 0.01% pimozide. Activity was significantly decreased during and Significantly increased following the pimozide diet. ($P<0.01$)

days after withdrawal of pimozide, activity increased to 150 to 200% of control activity.

Relationship of the Duration of a Haloperidol Containing Diet to the Development of Withdrawal Hyperactivity

Groups of 4 mice were fed a diet containing 0.005% haloperidol for periods of 1,2,6 or 10 days and then placed on a control diet. Activity measured on the night of the second day after withdrawal of the haloperidol diet was significantly below control after the 1 day diet and not significantly different from control after the *3* day diet (Fig. 3). Two days after the 6 day diet of haloperidol, activity was nearly 200% of control. The degree of withdrawal hyperactivity did not increase further following cessation of a 10 day diet of haloperidol.

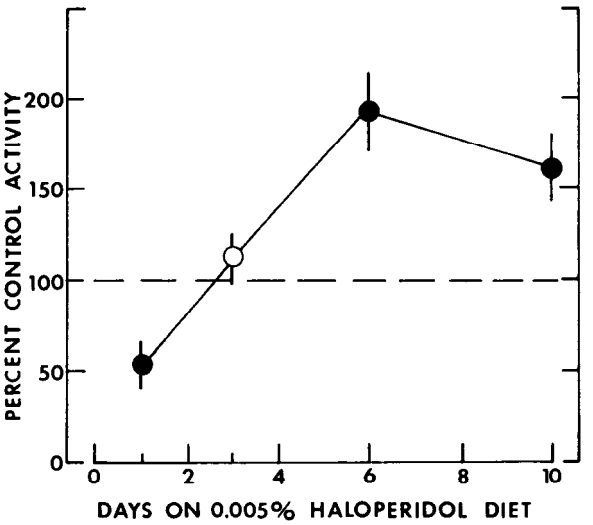


Figure 3

Figure 3. Relationship of the duration of feeding of a haloperido1 diet to the development of withdrawal hyperactivity Groups of 4 mice were fed a diet containing 0.005% haloperidol for periods of l, 3, 6, 10 days and were then fed a control diet. The circles represent total nocturnal activity starting on the second day after withdrawal of haloperidol expressed as percent of initial control activity (N=3). Solid symbols indicate a significant difference from control at P<0.01.

Apomorphine-Induced Motor Activity and Rearing Behavior Following Cessation of Chronic Diets of Haloperidol

If the decrease of locomotor activity during the administration of haloperidol results from dopaminergic receptor blocking actions of this drug, then the observed increase in spontaneous locomotor activity following withdrawal of the haloperidol-containing diet may be due to enhanced sensitivity of central dopaminergic neurons. If this is true, the locomotor activity response to apomorphine, a direct acting dopaminergic agonist, should also increase. To test this hypothesis, mice were fed a diet of 0.005% haloperidol for 10 days, and the response to apomorphine or vehicle was determined on the second day of withdrawal (day 12). Apomorphine, at a dose of 0.25 mg/kg, did not alter activity in control mice but produced significant locomotor stimulation in the haloperidol-treated group (Table 1). The result is compatible with an increased sensitivity of dopaminergic receptors. At higher doses of apomorphine, the degree of locomotor stimulation was similar in the control and the haloperidol-treated mice.

TABLE 1

Apomorphine-induced locomotor activity following a chronic diet containing 0.005% haloperidol

Dose of Apomorphine (mg/kg, s.c.)	Counts/30 min	
	Diet: Control	Haloperidol
0.25	-270 ± 78	1450± 94
0.50	1820±140	2463± 85
1.00	2728±135	2607±180

At 48 hours after withdrawal from a 10 day control or haloperidol-containing diet the mice were accommodated to the test environment for 30 min prior to receiving either 0.1% sodium metabisulfite or apomorphine s.c. The values represent apomorphine-stimulated activity (mean ± SEM for 6 determinations). Underlined values indicate a significant difference from control at P<0.01.

In another experiment, the effects of apomorphine on rearing behavior were determined on the third day following a 5 day diet of haloperidol. Mice previously fed haloperidol exhibited significantly greater rearing activity in response to apomorphine at doses of 0.25 and 0.50 mg/kg, than did control mice (Fig. 4).

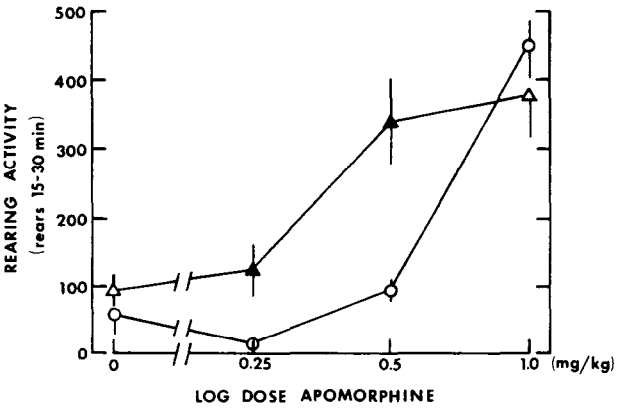


Figure 4

Figure 4. Apomorphine-induced rearing activity in mice following withdrawal of a diet containing haloperidol. Rearing activity of groups of 4 mice was determined as described in METHODS. At 3 days following 5 days of a control diet (○) or a diet containing 0.005% halperidol (△), the mice were accommodated to the test environment for 30 min prior to receiving either 0.1% sodium metabisutphite or apomorphine s. c. The data represent the number of rears during the period of 25 to 30 minutes after injection (mean ± 1 S.E., N=6). Solid symbols indicate significant differences from control at P<0.01.

Effects of Dopaminergic Agonists on Gnawing Behavior of Mice After Cessation of a Chronic Diet Containing Haloperidol

The ability of various dopaminergic agonists to produce gnawing was tested in control mice and in mice 2 days after a 5 day diet containing 0.005% haloperidol. The results are summarized in Table 2. A dose of 30 mg/kg apomorphine was required to elicit gnawing in a majority of control mice, while at all doses tested apomorphine caused gnawing in mice previously receiving a haloperidol-containing diet. At the doses tested, piribedil and L-DOPA caused significant gnawing only in the haloperidol-treated mice. The gnawing responses to d-amphetamine and methylphenidate were more pronounced in the haloperidol-treated group. The enhanced sensitivity to d-amphetamine was also evidenced by the fact that all mice previously on the haloperidol-containing diet died after 8 mg/kg of d-amphetamine while there were no deaths in the control group. Benztropine, which has dopamine uptake-blocking and anticholinergic properties, did not induce gnawing in either control or haloperidol-pretreated mice.

TABLE 2

Effects of dopaminergic agonists on gnawing behavior of mice after a chronic haloperidol-containing diet

Treatment (mg/kg)	Oiet	
	Control	Haloperidol
Apomorphine HCl (s.c.)		
2.5	0/6	6/6
5	2/6	6/6
10	0/6	6/6
20	1/6	5/6
30	5/6	6/6
Piribedil (s.c.)		
10	0/6	2/6
20	0/6	3/6
40	0/8	6/8
d-Amphetamine sulfate (i.p.)		
	0/7	4/7
	1/5	3/5
6	3/6	6/6*
16	6/6	---
L-DOPA (i.p.)		
	0/6	0/6
400	0/6	3/6
800	1/7	6/7
Methylphenidate (i.p.)		
	2/18	10/12
30	3/7	6/6
Benztropine (i.p.)		
	3/6	0/6
20	0/5	0/5
40	0/5	0/5

Mice were placed on a diet containing 0.005% haloperidol for 5 days and then fed a control diet for 2 days. Pairs of mice were then injected with the various dopaminergfc agonists, placed in a box containing a corrugated cardboard floor for 1 hour. and the presence or absence of gnawing was recorded (see METHODS and [14]. Data represent ratios of the number of pairs tested. exhibiting gnawing'to the total number of pairs of mice tested. Underlined ratios indicate those values that are significantly different from control (p<.01). Thirty minutes prior to the injection of L-DOPA. 50 mg/kg of MK-486. a peripheral decarboxylase inhibitor, was administered (i.p.) to the mice.
*, all twelve mice died within 3 hours after the injection of d-amphetamine.

DISCUSSION

Prolonged blockade of dopaminergic receptors with neuroleptics appears to cause development of supersensitivity of the receptors; there is considerable recent behavioral evidence to support this view. Following withdrawal of chronic treatment with neuroleptics, dopamine agonists produce stereotyped behavior at doses lower than required in control animals [6,8,12,17,19,23]. The present study demonstrates that in addition to stereotyped behavior, several other behaviors in mice are altered following cessation of chronic diet of haloperidol. Such mice exhibited increased spontaneous locomotor activity, increased apomorphine-stimulated locomotor activity and rearing and increased sensitivity to dopaminergic agonist-induced gnawing behavior.

Asper et al. [1] and Møller-Nielsen et al. [13] found that, after repeated administration of haloperidol and other neuroleptics, marked tolerance developed toward the ability of these drugs to inhibit apomorphine-induced stereotyped behavior. The development of tolerance may reflect receptor supersensitivity before withdrawal of the neuroleptic. The failure to demonstrate development of tolerance in the present study is possibly due to administration of a receptor-saturating dose of haloperidol.

The fact that sensitivity to apomorphine is altered supports the view that the major synaptic change involved is at the receptors. This is also consistent with earlier studies of supersensitivity of central dopaminergic responses to apomorphine following 6-hydroxydopamine [16,20,21,22]. The supersensitivity following chronic alpha-methyltyrosine administration [2,5] also likely involves a change at the receptors, since the dose-response curve for apomorphine-stimulated locomotor activity in mice after a chronic diet of alpha-methyltyrosine is shifted to the left [10].

Fjalland and Møller-Nielsen [6] demonstrated that following chronic administration of haloperidol, methylphenidate induced gnawing at lower doses than required in control mice. The present study confirms and extends this finding to include the dopaminergic agonists, apomorphine, piribedil, L-DOPA and d-amphetamine. In rats, piribedil caused weak stereotyped behavior only after prior treatment with 6-hydroxydopamine [9], a finding consistent with the effects of piribedil in this study.

A transient, enhanced sensitivity to dopaminergic agonists has been clearly demonstrated upon withdrawal of chronic treatment with neuroleptics in animals. The increased spontaneous motor activity in these animals may be due to hyperactivity of dopaminergic neuronal systems in the brain. Increased activity of central dopaminergic neuronal systems in humans after termination of chronic neuroleptic therapy may be reflected by a variety of abnormal involuntary movements. Klawans and Rubovits [12] and Gerlack et al. [7] proposed that tardive dyskinesias, which are observed in patients on long term therapy with neuroleptics after reductions in dosage or discontinuation, may be due to dopaminergic hypersensitivity. In contrast to the often prolonged tardive dyskineasias, the time course of the enhanced motor responses in mice was short-lived, correlating more closely with the time course of mild, reversible oral-facial dyskinesias in adults following abrupt withdrawal of phenothiazines or haloperidol [3,11], and that of reversible choreiform-like involuntary movements of the extremities in children after withdrawal of neuroleptics [15].

ACKNOWLEDGMENT

The excellent technical assistance of Nan Friedle is gratefully acknowledged. (Supported by USPHS grant NS-090174.)

REFERENCES

1. Asper, H., M. Baggiolini, H.R. Burki, H. Lauener, W. Ruch and G. Stille. Tolerance phenomena with neuroleptics: Catalepsy, apomorphine sterotypy and striatal dopamine metabolism in the rat after single and repeated administration of loxapine and haloperidol. Eur. J. Pharmacol. 22:287-294, 1973.

2. Beuthin, F.C., T.S. Miya, D.E. Blake and W.F. Bousquet. Enhanced sensitivity to noradrenergic agonists and tolerance development to alpha-methyltyrosine in rat. J. Pharmacol. Exp. Ther. 181:446-456, 1972.

3. Crane, G.E. Persistent dyskinesia. Brit. J. Psychiat. 122: 395-405, 1973.

4. Creese, I. Behavioral evidence of dopamine receptor stimulation by piribedil (ET495) and its metabolite S584. Eur. J. Pharmacol. 28:55-58, 1974.

5. Dominic, J.A. and K.E. Moore. Supersensitivity to the central stimulant actions of adrenergic drugs following discontinuation of a chronic diet of alpha-methyltyrosine. Psychopharmacologia 15:96-101, 1969.

6. Fjalland, B. and I. Møller-Nielsen. Enhancement of methylphenidate-induced stereotypes by repeated administration of neuroleptics. Psychopharmacologia 34:105-109, 1974.

7. Gerlack, J., N. Reisly and A. Randrup. Dopaminergic hypersensitivity and cholinergic hypofunction in the pathophysiology of tardive dyskinesia. Psychopharmacologia 34:21-35, 1974.

8. Gianutsos, G., R.B. Drawbaugh, M.D. Hynes and H. Lal. Behavioral evidence for dopaminergic supersensitivity after chronic haloperidol. Life Sci. 14: 887-898, 1974.

9. Goldstein, A. Biostatistics: An Introductory Text, p. 272, MacMillan, New York, 1972.

10. Gudelsky, G.A., J.E. Thornburg and K.E. Moore. Blockade of alpha-methyltyrosine-induced supersensitivity to apomorphine by chronic administration of L-DOPA. Life Sci. (in press).

11. Jacobson, G., R.J. Baldessarini and T. Manschreck. Tardive dyskinesia associated with haloperidol (in press), 1974.

12. Klawans, H.L. and R. Rubovits. An experimental model of tardive dyskinesia. J. Neural Trans. 33:235-246, 1972.

13. Møller-Nielsen, I., B. Fjalland, V. Pedersen and M. Nymark. Pharmacology of neuroleptics upon repeated administration. Psychopharmacologia 34:95-104, 1974.

14. Pedersen, V. and A.V. Christensen. Antagonism of methylphenidate-induced stereotyped gnawing in mice. Acta Pharmacol. (Kbh.) 31:488-496, 1972.

15. Polizoes, P., D.M. Engelhardt and S.P. Hoffman. CNS consequences of psychotropic drug withdrawal in schizophrenic children. Psychopharmacol. Bull. 9:34-35, 1973.

16. Schoenfeld, R.I. and N.J. Uretsky. Altered response to apomorphine in 6-hydroxydopamine-treated rats. Eur. J. Pharmacol. 19:115-118, 1972.

17. Tarsy, D. and R.J. Baldessarini. Pharmacologically induced behavioral supersensitivity to apomorphine. Nature New Biol. 245:262-263, 1973.

18. Tarsy, D. and R.J. Baldessarini. Behavioural supersensitivity to apomorphine following chronic treatment with drugs which interfere with the synaptic function of catecholamines. Neuropharmacology 13:927-740, 1974.

19. Thornburg, J.E. and K.E. Moore. Supersensitivity to apomorphine ET495 (1-[3,4-(methylenedioxybenzyl)-4-(2-pyrimidyl]piperazine) and L-DOPA following intrastriatal 6-hydroxydopamine administration. Pharmacologist 15:254 (ABS), 1973.

20. Thornburg, J.E. and K.E. Moore. Supersensitivity to dopamine agonists following unilateral 6-hydroxydopamine-induced striatal lesions in mice. J. Pharmacol. Exp. Ther. 192:42-44, 1974.

21. Ungerstedt, U. Postsynaptic supersensitivity after 6-hydroxydopamine-induced degeneration of the nigro-striatal,dopamine system. Acta Physiol. Scand. Suppl. 367:69-93, 1971.

22. Von Voigtlander, P.F. and K.E. Moore. Turning behavior of mice with unilateral 6-hydroxydopamine lesions in the striatum: Effects of apomorphine L-DOPA, amantadine, amphetamine and other psychomotor stimulants. Neuropharmacology 12:451-463, 1973.

23. Von Voigtlander, P.F. Behavioral and biochemical investigation of dopamine supersensitivity induced by chronic neuroleptic treatment. Fed. Proc. 33:578 (ABS), 1974.

AUTHORS

J.E. Thornburg, Ph.D. and K.E. Moore, Ph.D.: Department of Pharmacology, Michigan State University, East Lansing, Michigan 48824

BLOOD PLATELET MONOAMINE OXIDASE ACTIVITY IN ANERGIC SCHIZOPHRENICS

Edward G. Shaskan, Robert E. Becker

INTRODUCTION

Throughout the history of biological research in schizophrenia there have existed numerous claims for peripheral (blood or urine) biochemical markers for this disease [16]. A consistant, albeit discouraging experience among investigators working in this field has been. the frequency, indeed the predictability, by which so-called biological markers of schizophrenia have not stood up under replication studies and which have often been refuted [16].

Recent twin studies and adoptee studies have provided data indicating that between 25% to 38% of monozygotic twins become concordant--i.e., both ill--for schizophrenia at some time during the period of high risk from age 15 to 45 [10]. Nine percent of children of biologically schizophrenic parents adopted-out near birth become schizophrenic as adults [12]. This data forms the core of the present consensus that certain factors predisposing to schizophrenia are genetically, probably polygenetically, determined.

In recent investigations from laboratories at NIMH, a highly significant reduction in the activity of blood platelet monoamine oxidase (MAO) has been reported for both chronic and acute schizophrenic patients as compared to controls [7,14]. The reduction in platelet MAO activity did not appear to be a product of the schizophrenic illness nor influenced by drug treatment [7,14; Dr. Dennis Murphy, personal communication]. This reduced blood platelet MAO activity may provide a genetic marker for vulnerability to schizophrenia [9,14].

The widely quoted findings of Murphy and Wyatt and their collaborators at NIMH [7,14] provide enticing implications for the understanding of the etiology of schizophrenia, if a relationship between peripheral and central MAO activity can be demonstrated. Preliminary investigations, however, have not suggested a reduced MAO activity in the brains of schizophrenics at autopsy [3,13]. It has been suggested that an excessively activated dopaminergic system in the brain may be etiologically important in some types of schizophrenia [4,9]. Once again, however, the finding that human platelet and brain monoamine oxidases are most dissimilar with respect to the

oxidation of dopamine [6,11] suggests that reduction of platelet MAO activity may have little relevance to the oxidation of that biogenic amine most relevant to schizophrenia [5].

Murphy (personal communication) now questions the hypothesis that low blood platelet MAO activity is a genetic marker for vulnerability to schizophrenia. He has modified his original hypothesis by excluding acute schizophrenic reactions (Carpenter, Murphy and Wyatt, submitted for publication). In this latest work, however, they do not question the basic relationship of low platelet MAO activity found in their chronic schizophrenic patients.

As part of a continuing psychopharmacologic study of anergic schizophrenia, we have now completed a study of twenty-four depressed schizophrenic outpatients treated with combined antipsychotic and antidepressant medication. As a working hypothesis, we predicted that lower MAO activity might sensitize the schizophrenic patient to the induction of psychotic symptoms by tricyclic antidepressants [1]. As two criteria for this effect of tricyclic antidepressant drugs in schizophrenics, we expected that those schizophrenics who reacted adversely as a group would show 1) an intensification of primary and secondary schizophrenic symptoms and 2) would have low MAO activity significantly different from non-reactors and/or controls. This paper reports preliminary work which is part of a larger study designed to replicate the findings of Wyatt and Murphy [7,14] and to test our hypothesis that schizophrenic patients with low platelet MAO activity will show increased vulnerability to induction of psychosis by tricyclic antidepressants.

MATERIALS AND METHODS

Twenty-six anergic schizophrenic outpatients were admitted to the study at the University of Connecticut McCook Hospital Psychiatric Service. Diagnoses of schizophrenia with depression were established before referral and confirmed by one of us (R.E.B.). All patients had a prior hospitalization with a discharge diagnosis of schizophrenic reaction and had evidence of a thought disorder recorded in the clinical record of their hospitalization. All patients had received antipsychotic medications prior to admission into this study. Ten hospitalized acute alcoholics free of organic neurological sequelae and seven laboratory personnel volunteered as controls.

Patients were free of psychoactive medication for two weeks and then blindly treated with either chlorpromazine (Thorazine, SKY) 100-1200 mg daily and imipramine (Tofranol, Geigy) 150 mg daily or thiothixine (Navane, Roerig) 5-60 mg daily and placebo. Blood platelet MAO activity was evaluated following the drug-free period and once weekly for four weeks on medication.

Platelet preparation from venous blood collected by vacutainer into 18 ml tubes anti-coagulated with acid-citrate-dextrose (Becton-Dickinson #4798) followed the method of Wyatt and co-workers [15] with the following modifications. Vacutainers containing the blood were placed in crushed ice and transferred to the laboratory (maximum time on ice equalled two hours) and the vacutainers were centrifuged at 4" C for ten minutes at 170g. The platelet-rich plasma supernatant was transferred to 10 ml polypropylene centrifuge tubes using plastic disposable pipettes. This procedure was repeated at 380g and 680g. The polypropylene centrifuge tube containing between 5 to 9 ml of platelet-rich plasma (PRP) was centrifuged again at 380g for 10 minutes to remove incidental contamination of the PRP by red cells. The PRP supernatant from the latter centrifugation was then transferred to a clean polypropylene centrifuge tube and centrifuged at 10,000g for ten minutes, after which the platelet-poor plasma was discarded. The platelet pellet was washed twice successively with 2 ml of saline, drained, and stored for up to 30 days at -60° C without measurable loss of MAO activity. Two-day old blood-platelet concentrates (approximately 300 ml of PRP) obtained from the American Red Cross Blood Bank were pooled and approximately 30 platelet pellets were prepared in centrifuge tubes and stored at -60°C as above.

Platelet pellets were sonicated in 0.1 ml of 0.05 M sodium-potassium phosphate ($Na\text{-}K\text{-}PO_4$) buffer, pH 7.2 per ml of

final PRP using a Kontes Cell Disrupter with power setting at number 3 and frequency setting between numbers 3 and 4. Complete disruption of platelet membranes was effected in one minute, as demonstrated by oil-immersion light-microscopy. Using C^{14}-tryptamine (1.25mC/mmole; 1.6 x 10^{-5} M, final concentration) as substrate, platelet MAO activity was linear for up to about 30 minutes and to about 0.4 mg of Lowry protein when incubated at 37°C in 0.5 ml of 0.05 M Na-K-PO_4 buffer, pH 7.2. In preliminary experiments we found the pH optimum value for human blood platelet MAO activity to be approximately pH 7.8. However, since platelet MAO activity assayed at pH 7.2 was about 91% of the activity found at pH 7.8 and since platelet MAO values from other laboratories [7,14] were assayed at pH 7.2, incubation conditions for MAO values reported here were at pH 7.2, at 37°C, and for 20 minutes. Typically, reaction mixtures contained platelet sonicates representing between 0.1 to 0.2 mg'of Lowry protein. The reaction was stopped by injecting 0.1 ml of 30% perchloric acid and the glass-stoppered centrifuge tubes remained on ice for 20 minutes, after which time 5 ml of toluene was added to each tube. The glass-stoppered centrifuge tubes were shaken for 10 minutes and centrifuged for 10 minutes at 2000g to separate the phases. Three ml of the toluene phase was then transferred into scintillation vials to which 10 ml of scintillation cocktail was added. Radioactivity of the samples was determined in ISOCAP-300 (Searle Analytic, Inc.) scintillation spectrometer with efficiencies for our labelled-samples ranging between 87 to 89%.

Red Cross platelet pellet samples were routinely included in every assay as a control for possible changes in MAO activity due to incidental technical causes. Boiling at 100°C for 10 minutes of the Red Cross blood platelet samples prior to adding the radioactive substrate served as the enzyme blank values for the assay.

RESULTS

Of the twenty-six patients admitted to the study, twenty-four completed the two-week washout and twenty-two patients completed the four weeks on-drug period. The mean age of the schizophrenic patients was 39.2 ± 3.8 years. This compared to a mean age of 44.6 ± 3.6 years for the alcoholic patient controls and 34.9 ± 2.5 years for the non-patient controls.

Blood platelet MAO activity values in our sample of anergic schizohprenic outpatients were bimodally distributed and suggested a natural division into two groups (Table 1). Arbitrarily, those individuals having a mean (minimum of three and maximum of five weekly blood samples) platelet MAO activity value of less than 2.99 mumoles/mg protein/hour are referred to as the "LO-MAO" group, while those individuals whose mean platelet MAO activity was above this value are included in the "HI-MAO" group (Table 1). Although the sample size for both alcoholic and non-patient controls was respectively smaller as compared to the schizophrenics, a similar bimodal distribution for these control subjects could also be discerned (Table 1). No striking correlation between subjects' sex and the bimodal distribution of blood platelet MAO activity was apparent (Table 1).

TABLE 1

DISTRIBUTION OF PLATELET MONOAMINE OXIDASE ACTIVITY IN SCHIZOPHRENICS AND CONTROLS

Blood Platelet MAO Values*	Numbers of Subjects (Sex) Schizophrenics	Alcoholics	Non-patients
1.00 - 1.49	2 (M,F)	1 (F)	0
1.50 - 1.99	5 (4M,F)	4 (4M)	2 (M,F)
2.00 - 2.49	5 (3M,2F)	1 (M)	1 (M)
2.50 - 2.99	1 (M)	1 (M)**	2 (2M)**
3.00 - 3.49	4 (3M,F)	0	0
3.50 - 3.99	2 (M,F)	2 (M,F)	1 (M)
4.00 - 4.49	4 (M,3F)	1 (F)	0
Above 4.50	1 (F)	0	1 (F)

*Mumoles product formed per hour per mg Lowry Protein.

'*Values of 2.75 and below were assigned to the "low" group.

Of the twenty-four schizophrenics who completed the washout and at least two weeks of the on-drug study, thirteen could be assigned to the LO-MAO group and eleven were assigned to the HI-MAO group (Table 2). The control subjects were also arbitrarily assigned on the basis of their platelet MAO activity to LO-MAO or HI-MAO groups. Thus, six

alcoholics and three non-patient volunteers had mean platelet MAO activity not significantly different from the mean platelet MAO activity of schizophrenics assigned to the LO-MAO group (Table 2). Similarly, four alcoholics and four non-patient volunteers had mean platelet MAO activity not significantly different from mean values for schizophrenic patients who had been assigned to the HI-MAO group (Table 2).

TABLE 2

MEAN BLOOD PLATELET MAO ACTIVITY

IN OUTPATIENT ANERGIC SCHIZOPHRENICS

AND CONTROLS

Subjects	Monoamine Oxidase Activity (mμmoles product/hr/mg protien) LO MAO	HI MAO	P Values
Anergic Schizophrenics	1.99±0.42(13)	3.78±0.65(11)	<0.001
Alcoholics	1.73±0.21(6)	3.62±0.69(4)	<0.001
Non-Patient -controls	2.04±0.30(3)	3.68±0.93(4)	<0.1

Table 2: Values represent the mean ± standard error of the mean for the number of individuals expressed in parentheses. One blood sample from each individual was assayed for MAO activity in triplicate. Mean MAO values for all subjects studied are not significantly different from each other within the LO-MAO or HI-MAO groupings.

It has been reported that blood platelet MAO activity correlates highly with increasing age in humans [8] and has a tendency towards higher activities in women than in men [8]. Also, platelet MAO activity has been shown to vary over the menstrual cycle in women with a mean variation of about 23% [2]. In this pilot sample our results suggest that neither. sex nor age appear to significantly correlate with the mean MAO activity for either the LO- or HI-MAO groups of schizophrenics (Table 3). Similarly, neither sex (Table 1) nor age appeared to be correlated with this high-low dichotomy in control subjects.

Since it has been suggested that blood platelet MAO activity might be a genetic marker for vulnerability to schizophrenia [7, 14], one might reasonably expect that primary symptoms of schizophrenia (thought disorder and flatness of affect) and secondary symptoms (hallucinations and delusions) would correlate with low platelet MAO activity. However, in our population of schizophrenics, no measures relating to primary schizophrenic symptomatology discriminated the LO- or the HI-MAO groups at either the beginning or the end of the study period. In a discriminate function analysis the hyperactivity score the Adjustment Scale (KAS) significantly distinguished between the LO- and the HI-MAO groups, with the mean value in the HI-MAO group being significantly (F=12.7) lower, thus less hyperactivity, than the mean value for the LO-MAO group (Table 3). The sleep disturbance score on the Hamilton Depression Scale (HDS) also discriminated at baseline between these blood platelet MAO groups (Table 3).

TABLE 3

RELATINSHIP OF SELECT PATIENT-VARIABLES

AT BASELINE TO MAO GROUPS

Variable	Mean Score LO-MAO	HI-MAO	F-Value	Qualitative Importance
Katz-Hyperactivity Score	3.6	2.1	12.7	Overhelming
HDS*Sleep Disturbance Score	1.8	1.0	5.0	Strong
Sex	9 male 4 male	5 male	4.0	Marginal
HDS Apathy Score	2.3	2.9	3.9	Marginal
Age	5 aged<35 8 aged<35	6 aged<35 5 aged<35	3.2	Weak

*HDS - Hamilton Depression Scale

Psychological testing was again performed on the twenty-two patients who completed the 4-week on-drug study, and analyses of covariance were run for some 66 psychological test variables as a two-factor analysis of covariance. The two factors were drug combination administered and platelet MAO group. As found upon discriminate analysis of primary schizophrenic symptoms at baseline, once again at the completion of the study, items such as "bizarreness" (KAS) and "thinking disorder" the Brief Psychiatric Rating Scale (BPRS) did not discriminate between LO- and HI-MAO groups (Table 4). The thinking disorder scores (BPRS) had a significant interaction with drug group, with chlorpromazine/imipramine better than thiothixene/placebo

(p<0.01, Table 4). Schizophrenic patients in the LO-MAO group, as compared to patients in the HI-MAO group, appeared significantly worse (lower score values) on the following items that are usually not considered to comprise primary or secondary schizophrenic symptomatology: HDS sleep disturbance (p<0.10), HDS somatization (p<0.05), BPRS anergia (p<0.05), and KAS hyperactiviy (p<0.05).

Imipramine was maintained at a constant dosage of 150 mg per day, while thiothixene and chlorpromazine dosages could be adjusted by the psychiatrist in charge (R.E.B.) as per clinical management of the patients. No significant differences in dosage between LO- and HI-MAO groups were observed for either thiothixene or chlorpromazine, although there was an apparent trend towards prescribing more chlorpromazine to patients in the LO- as compared to the HI-MAO groups.

TABLE 4

RELATIONSHIP BETWEEN SELECT CLINICAL VARIABLES

AND PLATELET MAO ACTIVITY OR DRUG THERAPY

	Adjusted Final Score				
	Thiothixene		Chlorpromazine/Imipramine		
Factor	LO MAO (N=7)	HI MAO (N=5)	LO MAO (N=5)	HI MAO (N=5)	Statistical Significance
Hamilton Depression Scale					
Sleep Disturbance	1.3	0.9	1.5	0.4	p<0.10 in favor of HI MAO groups.
Somatization	1.1	0.5	1.3	0.4	p<0.05 in favor of HI MAO groups.
"Psychotomimetic" Cluster	3.2	3.5	4.7	1.7	p<0.01 interaction between CPZ/Imip. and MAO groups
Brief Psychiatric Rating Scale					
Depression	2.3	2.6	2.8	1.9	None
Thinking Disorder	3.1	2.3	1.5	1.9	p<0.01 CPZ/Imip. significantly better than thiothixene.
Anergia	1.8	1.3	1.9	1.0	p<0.05 in favor of Hl MAO groups
Katz Behavior Inventory Scale					
Hyperactivity	3.7	2.4	2.9	2.4	p<0.05 in favor of HI MAO groups
Bizarreness	3.3	3.6	2.5	3.6	None

Table 4: "Psychotomimetic" cluster on the Hamilton Depression Scale is arbitrarily represented here as the mean sum of the following factors: Insight (#17), Diurnal Variation (#18), Depersonalization and Derealization (#19), Paranoid Symptoms (#20) and Obsessional and Compulsive Symptoms (#21).

Analyses for a total of 66 factors were run as a two factor analysis of covariance, the two factors being drug and MAO group. Final scores (at completion of study) were adjusted according to baseline (drug washout) scores. On all of the above rating scales, the lower the score the "better" the patient on any factor.

DISCUSSION

The studies by Murphy and colleagues [7,14] reporting a reduced blood platelet MAO activity in schizophrenic patients as compared to normal controls could not be replicated in a group of twenty-four schizophrenic outpatients. In our study individuals have either low or high platelet MAO activity, which is stable over a four-week period. The platelet MAO activity is independent of the diagnosis of schizophrenia. Low blood platelet MAO activity reported for chronic schizophrenics [7,14] may be an effect of chronic schizophrenic illness, hospitalization or a physiological

characteristic of some individuals independent of the schizophrenic illness per se, but disposing toward a chronic outcome. Primary and secondary schizophrenic symptomatology did not discriminate between LO- and HI-MAO groups within our schizophrenic outpatient population. Symptoms that relate more closely with affective states such as sleep disturbance, hyperactivity, anergia, and somatization may provide directions toward understanding the interaction of platelet MAO activity and schizophrenic illness (Tables 3 and 4). In an essentially replicative study we are presently evaluating blood platelet MAO activity and clinical symptomatology in a group of inpatient anergic schizophrenics. We are also performing a more systematic study using these variables on a larger group of non-patient volunteers and hospitalized alcoholics.

Triyclic antidepressants have been shown to induce schizophrenic symptoms in some anergic schizophrenic patients [1]. We postulated that low MAO activity patients would show an intensification of primary and secondary schizophrenic symptoms from treatment with a tricyclic antidepressant. Thinking disorder scores (BPRS), bizarreness scores (KAS) and secondary symptomatology did not appear to be worsened by tricyclic antidepressant (i.e., imipramine) treatment of our anergic schizophrenic patients (Table 4). We therefore do not have any direct evidence supporting this hypothesis.

ACKOWLEDGMENTS

The authors gratefully acknowledge the technical assistance of Mr. Kevin Norige and Ms. Gloria Grillo and thank Dr. Dennis Murphy and Ms. Cindy Donnelly for their helpful suggestions on the monoamine oxidase assay and interpretations.

We appreciate the help of Alfred J. Katz, M.D., and A. Matthew Gottlieb M.A., of the Connecticut Red Cross Blood Program, 209 Farmington Ave., Farmington, Connecticut.

REFERENCES

1. Becker, R.E. Evaluation of an amitriptyline-perphenazine combination in chronic schizophrenia. Amer. J. Psychiat. 127:827-831, 1970.

2. Belmaker, R.H., D.L. Murphy, R.J. Wyatt and D.L. Loriaux. Human platelet monoamine oxidase changes during the menstrual cycle. Arch. Gen. Psychiatry. 31:553-556, 1974.

3. Domino, E.F., R.R. Krause and J. Bowers. Various enzymes involved with putative neurotransmitters. Arch. Gen. Psychiat. 29:195-201, 1973.

4. Matthysse, S. Antipsychotic drug actions: a clue to the neuropathyology of schizophrenia? Fed. Proc. 32: 200-205, 1973.

5. Meltzer, H.Y. and S.M. Stahl. Platelet monoamine oxidase activity and substrate preferences in schizophrenic patients. Research Communications in Chemical Pathology and Pharmacology. 7(2):419-431, 1974.

6. Murphy, D.L. and R. Weiss. Reduced monoamine oxidase activity in blood platelets from bipolar depressed patients. Amer. J. Psychiat. 128: 1351-1357, 1972.

7. Murphy, D. and J. Wyatt. Reducted monoamine oxidase activity in blood platelets from schizophrenic patients. Nature. 238:225-226, 1972.

8. Robinson, D.S., J.M. Davis, A. Nies, C.L. Ravaris and D. Sylwester. Relation of sex and aging to monoamine oxidase activity of human brain, plasma, and platelets. Arch. Gen. Psychiat. 24:536-539, 1971.

9. Snyder, S.H., S.P. Banerjee, H.I. Yamamura and D. Greenberg. Drugs, neurotransmitters, and schizophrenia. Science, 184:1243-1253, 1974.

10. Stabenau, J. and W. Pollin. Experiential differences for schizophrenics as compared with their

non-schizophrenic siblings: twin and family studies. In: (eds.) Roff, M. and D. Ricks Life Historv Research in Psychopathology. The University of Minnesota Press, Minneapolis, 1970.

11. Tipton, K.F. Some properties of monoamine oxidase. Adv. Biochem. Psychopharm. 5:11-24, 1972.

12. Wender, P. The role of genetics in the etiology of the schizophrenias. Am. J. Orthopsychiatry. 39:447-458, 1969.

13. Wise, C.D. and L. Stein. Dopanine-beta-hydroxylase deficits in the brains of schizophrenic patients. Science. 181:344-347, 1973.

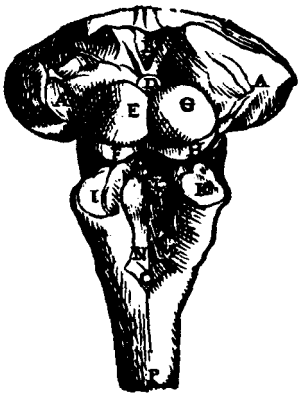
14. Wyatt, R.J., D.L. Murphy, R. Belmaker, S. Cohen, C.H. Donnelly and W. Pollin. Reduced monoamine oxidase activity in platelets: a possible genetic marker for vulnerability to schizophrenia. Science. 179:916-918, 1973.

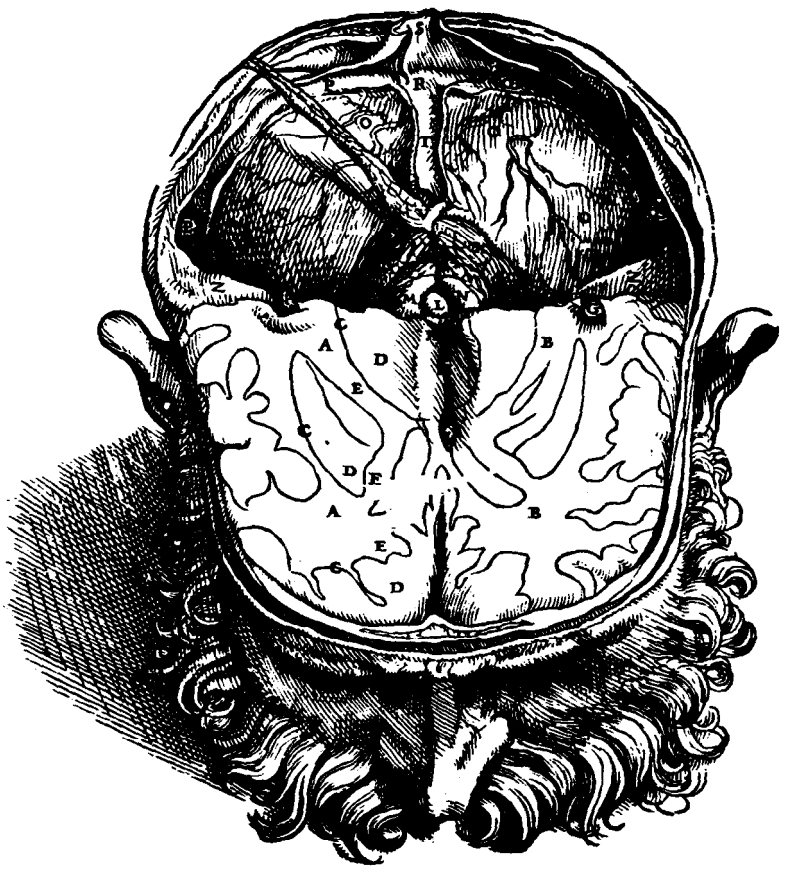
15. Wyatt, R.J., J.M. Saavedra and J. Axelrod. A dimethyltryptamine-forming enzyme in human blood. Am. J. Psychiatry. 130:754-760, 1973.

16. Wyatt, R., Termini and J. Davis. Biochemical and sleep studies of schizophrenia: A review of the literature - 1960-1970 - I. Biochemical studies. Schizophrenia Bulletin. 4:10-45, 1971.

AUTHORS

Edward G. Shaskan, Ph.D., Robert E. Becker, M.D.: Department of Psychiatry, University of Connecticut School Medicine Farmington, Connecticut 06032 (U.S.A.)


A
E
G
F


A
B
C
D
E
F

THE ROLE OF SEROTONIN AND NOREPINEPHRINE IN SLEEP-WAKING ACTIVITY

Peter J. Morgane, Warren C. Stern

INTRODUCTION

A wide variety of studies in the past decade have indicated that both the serotonergic neurons of the raphé system and catecholaminergic neurons, in particular those of the dorso-lateral pontine tegmentum, seem to play an essential, but by no means exclusive, role in the regulation of the sleep-waking cycle. These chemical systems regulate the vigilance continuum probably by modulating the activity of other neuronal systems and by interacting, directly or indirectly, with each other at several integrative levels in the brainstem. The view most widely put forward is that serotonin-containing nuclei and pathways play a more dominant role in regulating slow-wave sleep while activity in noradrenergic systems is involved in the generation of REM sleep. Since, however, these two chemical systems interact at several levels in the brain we will not only consider the evidence relating them individually to the states of sleep but also summarize their possible reciprocations in regulating the oscillations between the different states of vigilance.

THE ROLE OF CENTRAL MONOAMINE NEURONS IN THE REGULATION SLEEP AND WAKING: EVIDENCE AND OVERVIEW

The discovery of direct, probably monosynaptic, serotonergic and noradrenergic pathways to the cerebral cortex from the reticular formation of the brainstem [5,18,87,63] has greatly stimulated research on the role of monoamine neurons in sleep. An additional important factor has been the work of Jouvet [36,37,39], who discovered that monoaminergic drugs and lesions of the catecholamine and serotonin-containing nerve cell nuclei greatly interfere with particular facets of the sleep-wakefulness cycle.

In order to define the role of monoamines in sleep mechanisms the following three types of studies have usually been undertaken: (1) The effects of drugs (usually psychoactive) that alter monoamines on the cortical EEG; (2) The effects of specific lesions of the monoamine pathways to the telencephalon and diencephalon on the sleep-wakefulness cycle. This latter, presumably more specific, lesion approach has been made possible due to the introduction of 6-hydroxydopamine which, when injected into central catecholaminergic pathways, causes rather selective destruction of these fibers [85,87]. Also, 5,6-dihydroxytryptamine has been shown to exert somewhat selective actions on central serotonin pathways [6,33] and much research is now underway using these chemical lesioning tools in the evaluation of the relative roles of serotonin and norepinephrine neurons in sleep. However, the specificity of both of these neurotoxic agents is still in considerable doubt [69,16,75];

(3) Finally, studies have been made on the effects of sedative-hypnotic drugs and psychostimulant drugs on the relation of the sleep states to amine turnover in various dopamine, noradrenaline and serotonin pathways in the brain. Each of these three major categories of studies will be elaborated upon in this paper so as to typify the relations between biogenic amines and the sleep-waking states.

The definitive role of serotonin-containing neurons in the induction of slow-wave sleep and in the "priming" of REM sleep has been suggested predominantly by Jouvet's group [38] and is based on several varieties of converging experimental evidence. The following findings are most strongly persuasive in this regard: (1) The inhibition by para-chlorophenylalanine (PCPA) of the first step of serotonin synthesis results in total insomnia and this can be reversed to "physiological" sleep by subsequent injection of the serotonin precursor 5-hydroxytryptophan (5-HTP), which bypasses the inhibition of synthesis; (2) Lesions of the serotonin-containing neurons of the rostral and middle raphé system (especially the nucleus raphé dorsalis, nucleus centralis superior of Betcherew and nucleus raphé medialis) induce insomnia which is proportional to the extent of ablation of the serotonin-containing perikarya and to the decrease of serotonin in the forebrain at the level of the neuron terminals--this type of insomnia is not reversed by injection of 5-hydroxytryptophan; (3) Biochemical, and presumably selective, lesions of the serotonin terminals produced by the intraventricular injection of 5,6-dihydroxytryptamine result in a significant decrease of both slow-wave and REM sleep.

Our goal here is to discuss primarily the role of serotonin in sleep-waking mechanisms but, quite clearly, this cannot be done in isolation from the catecholinergic systems of the brain since there are obviously complex interactions occurring between these systems. Some of the evidence favoring the involvement of catecholaminergic systems in regulation of the vigilance states is as follows: (1) The inhibition of catecholamine synthesis at the level of tyrosine hydorxylase by alpha-methylparatyrosine has been shown to reverse the arousal and insomnia following the destruction of the rostral raphé system, thereby resulting in a state of sedation with cortical synchronization; (2) The destruction of the dorsal noradrenergic bundle in the mesencephalon is followed by a significant decrease of noradrenaline in the telencephalon and diencephalon and by a disturbance in arousal mechanisms both in waking and REM sleep; (3) Stirmula tion of catecholamine systems by d-amphetamine results in arousal.

The dopamine nigrostriatal pathways from the substantia nigra are not significantly involved with cortical EEG activity since quantitatively normal levels of polygraphic sleep and waking are seen after almost total ablation of the substantia nigra, following which there is practically complete depletion of striatal dopamine. The substantia nigra appears, however, to be concerned with the maintenance of waking activity since its destruction is followed by akinesia [34].

THE SEROTONIN-SLEEP HYPOTHESIS

We should now elaborate on the evidence for the role of serotonin in the regulation of the sleep states. The main experimental results which relate to the so-called serotonergic hypothesis of sleep have been the subject of several extensive reviews by Jouvet [37,38,40] and by Morgane and Stern [56]. The serotonergic hypothesis of sleep is an integral part of the general "monoamine theory" of the regulation of the states of vigilance. Its essence can be summarized as follows based, in large part, on Jouvet's earlier findings. Two antagonistic histochemically identified sleep and waking systems, whose cell bodies are located in the brainstem and whose terminals are dispersed widely in the brain, are involved in the alternation of the sleep-waking cycle. Serotonin-containing neurons are responsible for slow-wave sleep and for "priming" REM sleep, whereas ponto-mesencephalic catecholamine-containing neurons are involved in the "executive" mechanisms of REM sleep and in the maintenance of both tonic behavioral and EEG arousal. Each level of integration of the central nervous system is submitted to the synaptic action of these neurotransmitters, with the behavioral and EEG aspects of the sleep-waking cycle thus depending upon the organization of the post-synaptic efferent neurons. This monoamine

theory of sleep also states explicitly that REM sleep depends upon some "priming" serotonergic mechanism, and that slow-wave sleep and REM sleep must be in some way correlated. The theory emphasizes the crucial importance of the brainstem for the modulation of the sleep-waking cycle but also allows for the fact that other structures, primarily in the hypothalamus and in the basal forebrain area, are also intimately involved in the regulation of sleep-waking activity. Some supportive evidence for the hypothesis may be succinctly summarized as follows: (1) Inhibition of synthesis of serotonin with PCPA induces a secondary insomnia which correlates well with the depletion of brain serotonin. Further, this insomnia can be immediately reversed and "physiological" sleep is induced by the injection of a small dose of 5-HTP, which is rapidly converted into serotonin as shown by the subsequent relative increase of serotonin and 5-HIAA [8]; (2) The surgical destruction of serotonin-containing neurons of the midbrain and pontine raphk system induce a marked and protracted insomnia that is proportional to the decrease of serotonin in the terminal areas of the serotonin fiber pathways in the forebrain. This tybe of insomnia is not reversible by "secondary injection of a small dose of 5-HTP. no doubt due to the fact that the 5-HTP cannot be metabolized into serotonin since the serotonin cell bodies are destroyed and the terminals are secondarily degenerated. Both of these lines of data indicate quite clearly that neuropharmacological and neurophysiological alterations of serotonin synthesis lead to the same overall result, i.e., a significant decrease of sleep. There is reason to believe that pharmacological approaches, largely due to their non-specific effects, cannot give more precise information concerning the topography of the serotonin neuron systems involved in sleep control. As a result, several experimenters have recently chosen some more presumably specific techniques to alter the regional metabolism of serotonin neurons, either by chemically lesioning them with central injections of 5,6-dihydroxytryptamine or by indirectly increasing their metabolism with lesions of the dorsal noradrenergic pathway.

Jouvet, in particular, has marshaled evidence that groups of serotonin-containing neurons, located mostly in the rostra1 part of the raphé system, are responsible for the induction of sleep-- probably by releasing serotonin in strategic forebrain regions. Among these regions there is considerable evidence that preoptic serotonin terminals may Play a cardinal role in the induction of the post-synaptic events leading to cortical synchronization. Jouvet feels that this concept may reconcile, on both histochemical and biochemical bases, the two main theories that are currently proposed for the explanation of sleep, i.e., the "ascending" hypothesis related to the role of the serotonin-containing cells of the raphé [39] and the "descending" hypothesis related to the role of the preoptic area [59]. Any selective inactivation of serotonin neurons bearing upon the cell bodies located in the raphé, the serotonin ascending pathways in the medial fore-brain bundle, or the serotonin terminals located in the preoptic area itself might, therefore, be expected to lead to a significant decrease of sleep.

The activation of serotonin-containing neurons can theoretically be achieved through the direct stimulation of the serotonin cell bodies, as shown by the subsequent increase of 5-hydroxyindoleacetic acid in the terminals (2). However, this approach has given somewhat equivocal results upon the vigilance states, either increasing sleep in one study [48], or increasing waking in others [70,31]. Such opposing results might be due to the fact that electrical stimulation may activate other non-serotonin neurons in the raphé or may provoke the release of serotonin in some unphysiological manner. Overall, however, the bulk of the evidence favors the generation of arousal following raphé stimulation.

Jouvet has attempted to shed additional light on this problem by carrying out the activation of serotonin-containing neurons using neuropharmacological techniques. Thus, he has shown that the inactivation of catecholamine neurons with alpha-methylparatyrosine induces an increased turnover in serotonin neurons. In such instances the decrease of endogenous catecholamine is accompanied in the cat brainstem by an increase in the conversion of labeled tryptophan into labeled serotonin and by a significant increase of 5-HIAA [43]. If the hypothesis that catecholamine neurons inhibit serotonin neurons is true, then the destruction

of some known catecholamine pathways should induce some activation of the serotonin system.

Jouvet's group first approached this problem in the cat by a select lesion of the dorsal noradrenergic bundle [52]. Jones et al. [35] had shown rather conclusively that this bundle was involved in the maintenance of tonic cortical arousal. haeda et al. [52] found that bilateral lesioning of the dorsal noradrenergic bundle at the level of the isthmus immediately adjacent to the periaqueductal gray matter and to the rostral raphé system induced a most dramatic hypersomnia. They found an increase of up to 400% of REM sleep which was observed during the first day after the lesion. This remarkable degree of hypersomnia, at first characterized by a selective increase REM sleep and, secondarily, by an increase of both states of sleep, lasted for 8-10 davs. after which there was a slow return to baseline levels. Control lesions more dorsally in the colliculi or in the rostral midbrain did not induce any significant alterations of sleep. Only the caudal extension of the lesion into the cell groups of the locus coeruleus and locus subcoeruleus totally impaired this phenomenon by suppressing REM sleep. The mechanism of this most characteristic hypersomnia produced by far, the most pronounced increase of REM sleep yet observed experimentally and was analyzed by neuropharmacological and biochemical means, the results strongly suggesting that the serotonergic neurons of the raphé were involved.

Thus, the following points are most relevant: (1) The pretreamtment of cats with PCPA, which induces an almost total insomnia, prevents the subsequent hypersomnia which normally follows the destruction of the dorsal noradrenergic bundle; (2) When the biosynthesis of serotonin is measured during the maximum period of hypersomnia, i.e., 24 hours after the lesion, there is a significant increase in the conversion of labeled tryptophan into labeled serotonin in both the cerebral cortex and the raphé, while no alteration of endogenous serotonin or catecholamine can be detected; (3) Specific alterations of endogenous levels of monoamines have been detected in another series of experiments in which cats were sacrificed 8-10 days after the lesion [66]. In these experiments there was a decrease of norepinephrine in the forebrain (mostly in the cortex), which was explained by the destruction of the fibers essential to the maintenance of norepinephrine in these areas, i.e., the dorsal noradrenergic bundle. This decrease in norepinephrine was accompanied by a significant increase of both tryptophan and 5-HIAA levels in the brain, while serotonin did not change significantly. It would appear from these results that the turnover of serotonin neurons was still elevated 8-10 days after these lesions.

The specific mechanisms by which the serotonin-containing neurons of the raphé complex are activated have not yet been discovered. A possible explanation is that components of noradrenergic fiber systems ascending from the rostral part of the locus coeruleus might tonically control the activity of the serotonin-containing cell bodies in the raphé complex. Fluorescence mapping studies have shown that many catecholamine-containing terminals terminate close to the serotonin cell bodies in this area [12,51], but all criteria for a direct noradrenergic-serotoin synapse have not yet been met. If such a control exists, it is possible that lesions of noradrenergic fibers would release the activity of serotonin neurons as is suggested by the two biochemical controls used by the Jouvet group above.

We have recently [57] obtained preliminary electrophysiological evidence supporting the view that catecholamine neurons directly inhibit the activity of the anterior raphé cells. It was observed that electrical stimulation Of the locus coeruleus in anesthetized rats produced a long-lasting inhibition of spontaneous raphé unit activity (Fig. 1). Control recordings of units 1 mm lateral to the nucleus raphk dorsalis did not show this inhibition following locus coeruleus stimulation. Such a mechanism would provide much plasticity and flexibility in the influence of norepinephrine neurons (which are strongly implicated in the control of cortical arousal) upon the serotonin neurons involved in sleep.

In studies on the role of central serotonin neurons in sleep the relations to certain drugs affecting sleep should be mentioned. These can be summarized in general as follows: Barbiturates in sedative-hypnotic doses

FIGURE 1

INTER - SPIKE INTERVAL OF RAPHÉ UNIT FIRINGS AFTER BRAINSTEM STIMULATION

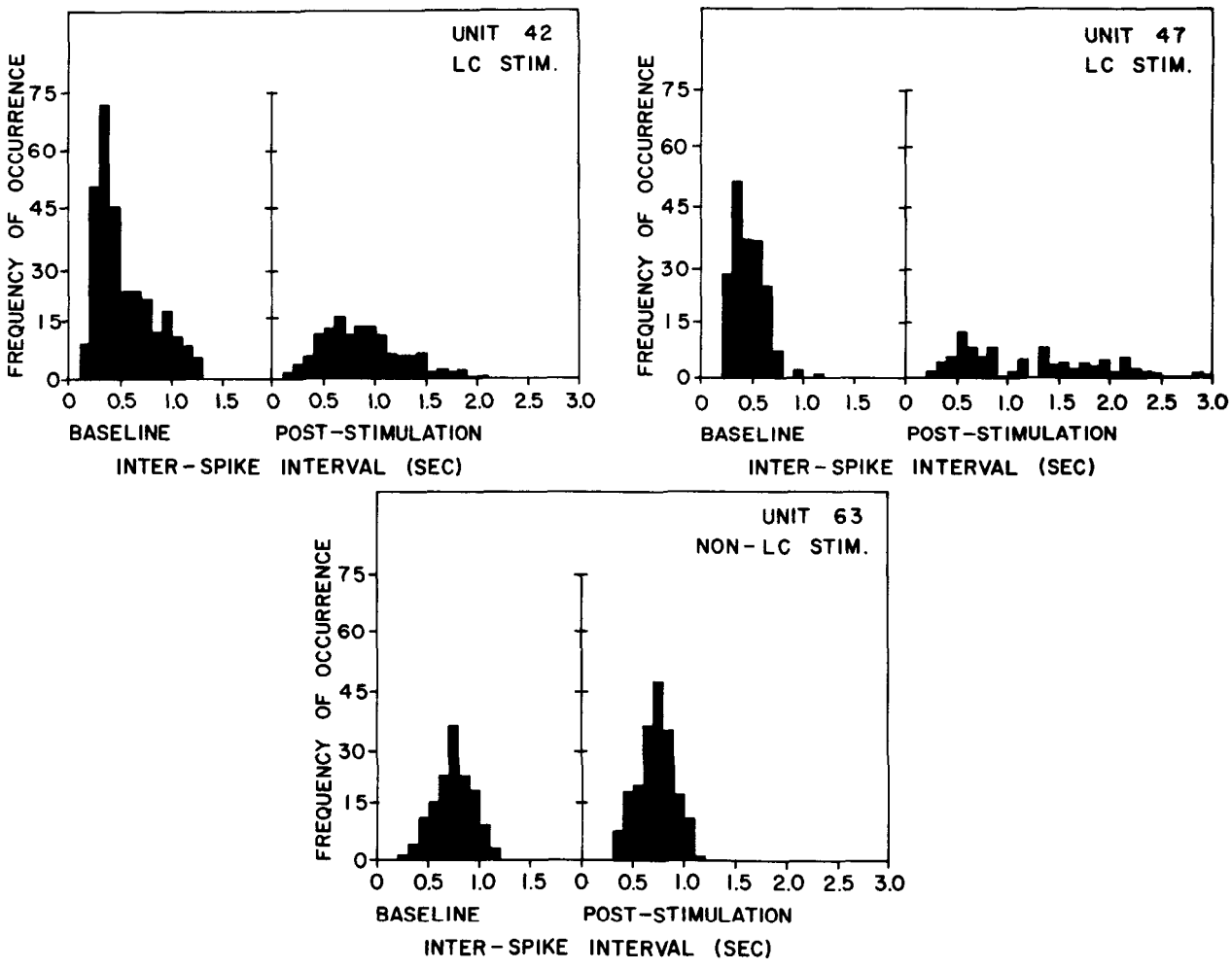


Figure 1.: Histograms of single unit activity in three raphé dorsalis neurons of rats in response to single pulse stimulation of the locus coeruleus (LC) or the adjacent reticular formation (non-LC stimulation). Rats were anesthetized with chloral hydrate, 350 mg/kg, i.p. and the LC was given a biphasic pulse of 20 A, 500 sec duration once every 30 sec for at least 5 min. In the base-line condition the interspike intervals (ISI) are clustered in the 0.5-1.0 sec. range, characteristic of raphé dorsalis spontaneous discharge rates. Following LC stimulation, however, there was a marked overall depression in the number of unit firings, illustrated by a decrease in the short ISIs and an increase in the long ISIS. The non-LC stimulation control produced no change in the distribution of raphé ISIs. (From Morgane, Stern and Berman, 57).

appear to decrease serotonin turnover in both ascending cortical and sub-cortical serotonin pathyways. It should be remembered that the ascending sub-cortical serotonin pathways to the hypothalamus originate mainly from the serotonin cell groups B7 and B8 (nucleus raphé dorsales and nucleus raphé medianus), whereas the cortical serotonin pathways run more laterally in the mesencephalon and appear to originate partly from cell group B9 [33]. The organization of the ascending serotonin neurons is therefore similar to that of the ascending norepinephrine neurons, with distinct cortical and sub-cortical pathways distinguishable (Fig. 2). Barbiturates appear to have a general depressant action, whereas minor tranquilizers may selectively depress activity in the cortical serotonin pathways which, as noted, are distinctly separated from the sub-cortical serotonin pathways. The turnover changes probably reflect changes in nervous activity in the pathways, since no direct action of these compounds on serotonin uptake and release have been discovered.

FIGURE 2

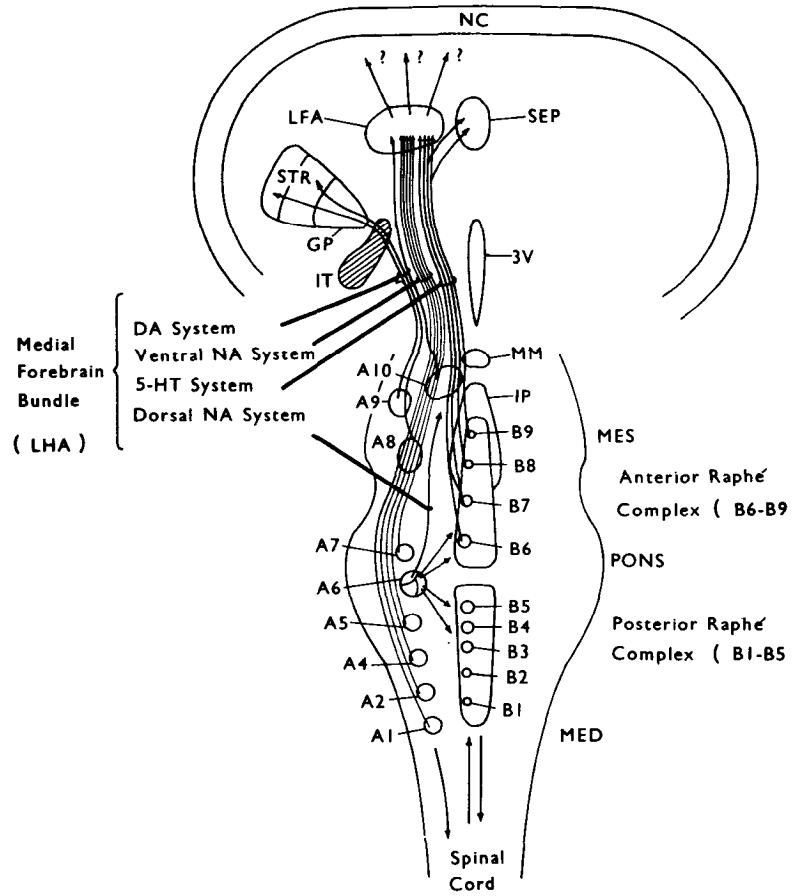


Figure 2.: Schema showing the principal monoaminergic pathways in the brain. Many of *the projections* of *these* aminergic *systems terminate in subcortical areas* such *as the hypothalamus, striatum, limbic system and basal forebrain areas. Other projections pass diffusely* into *several zones of the neocortex. The "A" and "B" coding systems on the diagram* are *the cell groups indicated in Dahlström* and *Fuxe (12). Abbreviations are as follows: MED: medulla; MES: mesencephalon; MM: mammillary nuclei; 3V: third ventricle; NC: neocortex; SEP: septum; LFA: limbic forebrain area; STR: striatum; GP: globus pallidus; DA: dopamine; NA: norepinephrine; 5-HT: serotonin; LHA: lateral hypothalamic area; IP: interpeduncular nucleus; IT: internal* capsule.

As reviewed above, Jouvet [42] has postulated that the ascending serotonin neurons are of critical importance especially for slow-wave sleep. The turnover studies of Lidbrink [50], revealing a dose-dependent decrease in the activity of the serotonin pathways by barbiturates and minor tranquilizers, do not, however, favor this view. It would seem that if the hypothesis of Jouvet is correct, the decrease in activity of the serotonin neurons would have to be visualized as a compensatory feedback response. Thus, the barbiturates and minor tranquilizers might induce slow-wave sleep by an action on or beyond the serotonin receptors and activity in the serotonin neurons might no longer be needed to induce slow-wave sleep. These results have been described in relation to changes in the firing rate of the raphé serotonin nerve cells [50].

THE ROLE OF CENTRAL CATECHOLAMINERGIC NEURONS IN SLEEP

The role of the catecholaminergic systems in relation to sleep-waking behavior can be outlined under three major headings:

1. The Effects of Monoamine-Modulating Psycho-Active Drugs on the Cortical EEG. Most monoamine-modulating drugs interfere with both dopamine and noradrenergic synapses. However, a few neuropharmacological tools have been developed which make it possible to interfere almost exclusively with activity in noradrenergic pathways. Additional drugs are known to block only dopamine and not noradrenergic receptors. By appropriate combinations of these agents Lidbrink et al. 1501 found that a decrease in wakefulness coincides with a maximal depletion of brain norepinephrine. They also speculated that newly synthesized norepinephrine is of particular importance for the maintenance of REM sleep. In other studies they showed that amphetamine caused EEG arousal and that the desynchronization produced by amphetamine was not blocked by dopamine receptor blocking agents such as spiroperidol, whereas haloperidol (which blocks both central dopamine and norepinephrine receptor sites) reduced the action of amphetamine on the cortical EEG. Their results were interpreted as underlining the important role of the coerulo-cortical (dorsal) noradrenergic neuron system as an arousal system in the brain in that they demonstrated that the effects of sedative-hypnotic drugs, such as barbiturates and minor tranquilizers, are mediated partly via depressing activity in this specific pathway.

With regard to psycho-stimulant drugs, amphetamine and related compounds have been found to cause release of dopamine and norepinephrine [19]. The cortical noradrenergic nerve terminals, however, appear to be particularly sensitive to these drugs. Furthermore, cortical norepinephrine turnover is increased and the decrease of cortical norepinephrine turnover found after barbiturates is counteracted by amphetamine. In view of the fact that amphetamine reduces hexobarbital sleeping time these results further demonstrate the important role that these cortical noradrenergic networks play in wakefulness.

2. The Effects of 6-Hydroxydopamine-Induced Lesions of the Dorsal Noradrenernic Pathway. 6-Hydroxydopamine was injected into the cortical noradrenergic bundle (dorsal noradrenergic pathway) at the level of the dorsomedial tegmentum of the caudal mesencephalon of rats by Lidbrink et al. [50]. This neurotoxic treatment resulted in interruption of nerve impulse flow in the dorsal noradrenergic pathway following which histochemical analysis showed an almost complete disappearance of cortical noradrenergic nerve terminals 15 days after the lesion. The interruption of the cortical noradrenergic pathway by 6-hydroxydopamine in these studies resulted in a 20% decrease of waking during 8 days of recording. REM sleep was also suppressed by 18% whereas there was no significant change in percent of slow-wave sleep after 6-hydroxydopamine. Thus, the effects appear to be transient, being most pronounced in the beginning of the period following 6-hydroxydopamine administration. No changes in waking and sleep were found in animals 25-30 days after chemical lesions of the dorsal (coerulo-cortical) noradrenergic pathway.

These studies of Lidbrink et al. [50] agree generally with the conclusions reached by Jouvet's group (see below). The latter group have underlined the role of the catecholamine-containing

group A8 in the ventro-lateral midbrain tegmentum in waking mechanisms. These findings were based on the results of lesions in this area but, since these lesions also damaged part of the ascending noradrenergic pathways, they could also be explained partly on the basis of interruption of these pathways. Lidbrink's group demonstrated the importance of the anterior dorsolateral portion of the locus coeruleus (so-called principal locus coeruleus), since this portion of the nucleus gives rise to the cortical noradrenergic pathway which was damaged in their experiments. Also, the recent studies of Henley and Morrison [26] showed that after bilateral lesions in the pontine tegmentum there resulted episodes of REM sleep in which the atonia of that state was absent. They concluded that the hypotheses, stating that the locus coeruleus or other isolated nuclei of the pons are specifically concerned with the initiation of REM sleep, are not supported by their findings. Their histological data appeared to show lesions largely confined to the locus coeruleus area.

3. The Effect of Psychoactive Drugs on Norepinephrine Turnover. With regard to the role of sedative-hypnotic drugs and psycho-stimulant drugs on amine turnover in various chemical pathways, it has been shown that barbiturates and minor tranquilizers cause, in sedative-hypnotic doses, a selective decrease in noradrenergic turnover in the coerulo-cortical noradrenergic pathway [11,50]. These experiments showed that the subcortical noradrenergic nerve terminals, having a different origin, are unaffected unless the noradrenergic turnover is accelerated by stress. Under circumstances of stress in rats these drugs slow down the turnover: of norepinephrine both in the hypothalamus and in the cerebral cortex. In studies of neuroleptics Lidbrink found that the sedation caused by these drugs is related to noradrenergic receptor blockage, which results in increased norepinephrine turnover [9,3,62]. These drug studies all show the importance of an intact neurotransmission in central noradrenergic neurons for the maintenance of wakefulness.

The literature on the effects of catecholamines on sleep is confusing because of two separate paradoxes or contradictions. First, there is the question of whether catecholamines in the brain produce sleep or arousal. There is evidence that direct brain application of norepinephrine epinephrine produce sleep or coma [53], yet a great deal of indirect evidence using amphetamines, precursors, etc., suggest that catecholamines have a definite role in waking. This disagreement might best be resolved at the moment in favor of the second alternative. It is clear that the reticular activating System, and catecholamine-containing neurons in it, are essential for waking, and that by increasing or decreasing catecholamine levels in a relatively physiological way, i.e., producing increases where they normally occur, this tends to induce wakefulness after increases, and drowsiness or sleep after decreases. The data suggesting that catecholamines produce sleep involve either direct brain application or administration of large doses to chicks or other immature organisms with poorly developed blood-brain barriers. Probably the results can best be explained as local and unphysiological effects of large quantities of amines entering the central nervous system. In fact, what is produced by direct application of the catecholamines is seldom "sleep" by EEG criteria, but rather behavioral inactivity or coma. Thus, it would appear that brainstem catecholamines play a major role in insuring and maintaining wakefulness.

There is another controversy about the role of catecholamines, in the two qualitatively distinct states of sleep, synchronized and desynchronized. Jouvet's group, as noted, has suggested, on the basis of indirect evidence, that norepinephrine is a neurohumor that produces or releases REM sleep. Hartmann [23] and we ourselves [82] have presented evidence for the opposite view, i.e., that low catecholamines are related to high REM time. This view has led to a theory of the function of REM sleep, one that accounts for both the relationship of catecholamines to REM and their effects in maintaining arousal. Hartmann [24] and Stern and Morgane [82] suggest that one of the biological functions of REM sleep is to maintain or restore the functional integrity of the catecholamine-dependent neuronal systems. The following arguments are cogent in this regard: It is important to consider the effects on sleep of drugs and other conditions associated with increases and decreases in brain catecholamine

levels. In human studies it seems clear that drugs such as amphetamines, monoamine oxidase inhibitors and the tricyclic anti-depressants, which are thought to produce their effects by increasing levels or availability of brain norepinephrine, all induce sharp decreases in REM time. Hartmann [23] notes a wide variety of drugs which, when first administered, produce a small decrease in REM time, but in his human studies the effects of these anti-depressants is far more marked. Furthermore, a great decrease in REM time is produced with very little or delayed rebound, as though, for a time at least,the drugs were "substituting" for REM time. The same effect has been shown for electroconvulsive shock in animals (also an "anti-depressant" situation associated with increased available catecholamines), i.e., greatly reduced REM time with little or no rebound. Also, in the clinical syndrome of mania (thought to be associated with highly available catecholamines) there was found to be a very low REM time, low even as a percent of the total of the lowered sleep. Thus, in many situations it seems that high central catecholamine levels appear to be associated with low REM time.

We can summarize the role of catecholaminergic systems in sleep-waking activity as follows:

A. Waking Mechanisms. The intervention of catecholaminergic mechanisms in the control of tonic cortical arousal in the cat is suggested by converging experimental evidence which is as follows: (1) The increase of cerebral catecholamine (after L-dopa) induces a long-lasting arousal in the cat; (2) The inhibition of the synthesis of catecholamines with alpha-methylparatyrosine decreases waking in normal cats and totally suppresses the behavioral and EEG arousal which normally follows the injection of d-amphetamine.

Norepinephrine, but not dopamine, seems to be involved in the maintenance of tonic cortical arousal for the following reasons: (1) The destruction of the substantia nigra, which decreases telencephalic and diencephalic (including striatal) dopamine levels by more than 90%, does not interfere significantly with cortical EEG arousal but strongly alters behavioral waking; (2) The destruction of the dorsal noradrenergic pathway in the mesencephalon, or of cell group A8 in the mesencephalic reticular formation, strongly reduces telencephalic and diencephalic norepinephrine and increases cortical synchronization. There is a significant correlation between the decrease of cortical arousal and the decrease of norepinephrine in the mesencephalon and the forebrain.

B. REM sleep. The intervention of catecholamine- and/or acetylcholine-containing neurons of the latero-dorsal pontine tegmentum in the "executory mechanisms" of REM are suggested by a variety of neuropharmacological and neurophysiological findings: (1) Reserpine-pretreated cats receiving dopa exhibit REM much earlier (2-4 hrs) than animals not receiving dopa. This suggests that the refilling of some pools in the catecholamine terminals could be a condition for the reappearance of REM. It is noteworthy that in our experiments in cats we were not able to observe any dopa-induced increase in REM time [81]; (2) Certain alpha-receptor blocking drugs, which cross the blood-brain barrier of the rat, were found to suppress REM; (3) The administration of alpha-methyldopa, which results in the synthesis of the false transmitter alpha-methylnorepinephrine, selectively suppresses REM in the cat for 12 to 16 hrs and decreases waking; (4) In Jouvet's studies, and in those of Henley and Morrison [26], the destruction of the caudal third of the nucleus locus coeruleus suppressed the motor inhibition, but not other components of the REM state, which occur during REM sleep. After such a lesion Jouvet's cats presented, periodically during sleep, some peculiar hallucinatory-like behavior (*rage*., defense against some imaginary enemy, etc.). Such behavior is good evidence that REM sleep may be accompanied by oneiric activity in the cat. The total destruction of the caudal part of the locus coeruleus and of the nucleus sub-coeruleus selectively suppressed all the central and peripheral components of REM sleep including PGO waves [39]. On the other hand Henley and Morrison [26] continued to observe what they consider to be REM sleep episodes for up to six months following locus coeruleus lesions. In chemical lesion studies selective suppression of REM was obtained secondarily after micro-injections of 6-hydroxydopamine which was thought to selectively destroy catecholamine neurons in

the latero-dorsal pontine tegmentum [30,67,90]. However, Panksepp et al. [64] found a significant elevation of REM following administration of this agent into this same approximate region.

ON THE ROLE OF CENTRAL DOPAMINE NEURONS IN SLEEP

Barbiturates and minor tranquilizers in hypnotic doses cause a decrease in turnover of dopamine in the neostriatum and the limbic forebrain areas [11]. Another drug known to induce sleep is gamma-hydroxybutyric acid [7] and this effect has been related to an abrupt rise of the dopamine levels in the neo-striatum [73]. This marked rise is similar to that seen after interruption of the dopamine pathway [4,61] and may, therefore, represent a dramatic decline in dopamine release, resulting in increased dopamine storage due to continued dopamine synthesis. It is also well known that the nigro-neostriatal dopamine pathway is essential for behavioral arousal [18,87]. It is, therefore, likely that the observed decreases in dopamine activity in the forebrain found after treatment with sedative-hypnotic drugs can mediate the behavioral depression found after these drugs are administered. It is possible that these effects are mediated by the meso-limbic dopamine pathways. Harner and Dorman [25] have shown that dopamine may be responsible for cortical activation and reported a consistent behavioral and electrographic arousal to result from L-dopa administration. Of course, in the normal state coordination must exist between the sleep-wakefulness cycle and behavioral activity and this involves, in all likelihood, complex interactions between different chemical systems in the brain. It would, thus, appear that the action of the sedative-hypnotic drugs may primarily be on the nervous pathways subserving sleep and wakefulness. When sleep is favored, the behavioral arousal system, such as the dopamine system to the neostriatum, would then be turned off. Similarly, when arousal is favored by activity in the dorsal noradrenergic pathway, the serotonin cells in the raphé may be inhibited.

In summarizing the various results above, it should be noted that: (1) The cortical noradrenergic pathway from the principal locus coeruleus appears to be important for the maintenance of wakefulness and REM sleep and that the effects of sedative-hypnotic drugs, such as barbiturates and minor tranquilizers, are partly mediated by a depressing activity on this particular pathway; (2) The activity of the cortical and subcortical serotonin pathway are also affected, directly or indirectly, by sedative and hypnotic drugs such as barbiturates and minor tranquilizers; (3) The activity of the ascending dopamine neurons, which are involved in behavioral and probably EEG arousal, is depressed by barbiturates and minor tranquilizers. This effect may be responsible for the behavioral depression seen after administration of these compounds. A suppression of the nigro-neostriatal dopamine neurons may be the way in which the neural substrate for sleep-wakefulness control can insure behavioral depression during sleep. Of course, many of these formulations are still tentative, but enough models of chemical neuron interactions have been derived to begin to test specific hypotheses regarding their role in the vigilance states and in drug action.

RELATIONSHIP OF THE BIOGENIC AMINES TO PHASIC EVENTS AND BEHAVIOR

Dement and his co-workers [13] have gathered data suggesting that serotonin is somehow involved in PGO discharge control. They have pointed out that in Jouvet's original investigations on serotonergic neurons he achieved pharmacological effects on serotonernic systems with acute administration of PCPA and reserpine and obtained anatomical specificity with stereotaxic lesions. An example of the reserpine effect on PGO occurrence in the cat is shown in Fig. 3. Both the pharmacological and stereotaxic approaches have led to insomnia or a great reduction in total sleep time and the emergence of PGO activity into the waking state. Dement's group [14] focused on the chronic effects of PCPA administration and found that this regimen does not appear to produce a direct pharmacological, effect, i.e., no behavioral response was observed in the immediate post-injection period. In their dose regimen PCPA did reduce serotonin concentrations in all parts of the brain to 0 to 10% of controls by the fifth PCPA-treatment day.

During the first 24 hrs after the beginning of PCPA administration, they

FIGURE 3

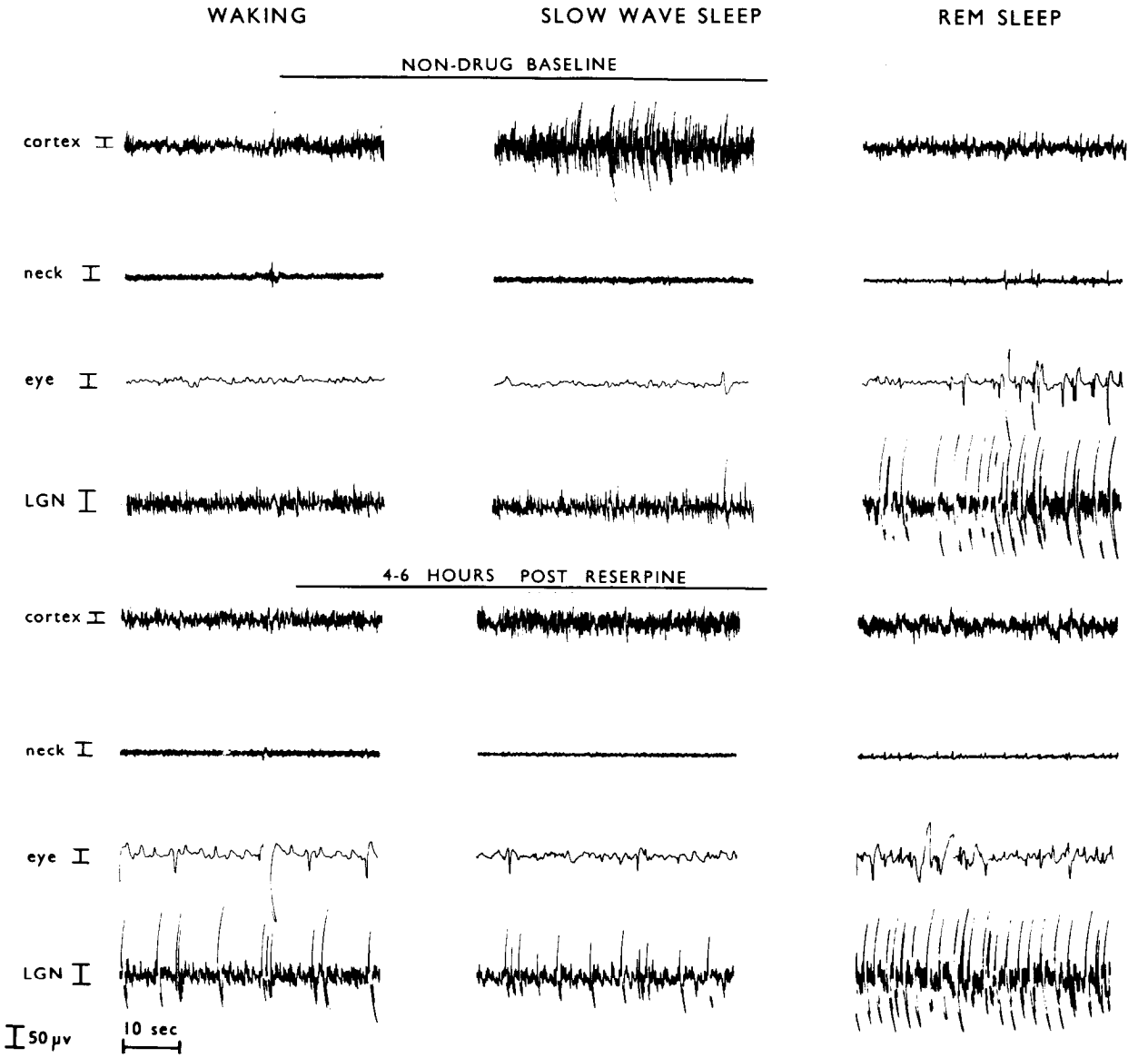


Figure 3: Polygraph write-out illustrating the effects of reserpine (Serpasil) 0.25 mg/kg, i.p. upon the occurrence of PGO spikes in the lateral geniculate nucleus (LGN) of a cat during waking, slow-wave and REM sleep. Cortical, neck and eye tracings are taken in order to identify the vigilance state. Note the large increase in the occurrence of monophasic PGO spikes in waking and slow-wave sleep following reserpine administration. (From Stern and Morgane, 81).

found that REM time generally stayed the same or slightly increased. Slow-wave sleep was somewhat reduced, dropping more rapidly on the second and third PCPA days. Near the end of the third day, total sleep time decreased precipitously and often reached zero for limited periods, the minima being generally seen on the fifth day of PCPA treatment. After two or three days of very low sleep values a marked recovery in total sleep time then began and included both REM and slow-wave sleep. Total sleep time (both REM and slow-

wave sleep) reached approximately 70% of the baseline values even during continued administration of PCPA. At the point where total sleep time began to increase, reorganization in the temporal sequencing of the sleep states become apparent. Normally in the intact cat, there is a marked tendency for the order of the sleep states to be wakefulness, followed by slow-wave sleep, then REM sleep. In the chronic PCPA cat, the order observed by Dement's group was most typically wakefulness, followed by REM sleep, and then slow-wave sleep.

The earliest changes in phasic activity during PCPA administration noted by Dement was an increase in the rate of PGO spikes during REM sleep and a decrease in the number of spikes that commonly precede REM periods. These changes become evident within 8-12 hrs after the first dose of PCPA. Later on they observed an emergence of PGO activity throughout slow-wave sleep and the waking state and such activity could be seen at each point in the pons-geniculate-occipital cortex circuitry. As this activity appeared in the waking state, the overall rate of spike discharge began to drop in REM sleep. Another change in the chronic PCPA cat was an increase in the intensity of muscular twitching. Finally, behavioral effects began accompanying PGO waves in the waking state, e.g., orienting behavior occurred in association with bursts of PGO waves.

During the period when PGO waves were discharging in the waking state, other inhibitory effects on these waves became apparent. Behavioral arousal or intense stimulation caused suppression of PGO waves. If the animal became frightened, the waves would temporarily disappear. In addition, L-dopa or amphetamine would totally suppress the PGO waves for variable lengths of time and 5-HTP totally reversed the release of PGO waves during wakefulness.

Thus, if it is assumed that a primary effect of PCPA is inhibition of serotonin turnover, then the earliest and possibly the most basic effect of this change is on the regulation of PGO spike discharge. Even after sleep returned PGO spike regulation was still disrupted. Furthermore, with respect to the neurophysiology of PGO control, McGinty et al. [55], using unrestrained, chronically recorded cats, studied unit activity in the raphé nuclei in relation to PCPA-induced PGO waves. They found that thirty percent of the units in the dorsal raphé exhibited regular and slow firing which was stable during a wide variety of waking behaviors and stimulus conditions, such regularity even continuing in slow-wave sleep. Clear cessations in the firing of these units occurred only during REM sleep and in slow-wave sleep preceding LGN waves. The specificity and consistency of these findings strongly suggests that the release of serotonin from the dorsal raphé neurons normally suppresses LGN waves of REM sleep.

In further attempts to confirm these findings, Dement's group has observed the effects of electrical stimulation of the raphé nuclei in cats with electrodes implanted in the nuclei raphé dorsalis, pontis and magnus. Stimulation of the nucleus raphé dorsalis during REM sleep caused complete suppression of geniculate PGO waves during the period of stimulation. These same stimuli were ineffective in suppressing PGO waves when delivered to the nuclei raphé pontis and raphé magnus. Stimulation of the nucleus raphé dorsalis in the waking state produced little or no discernable behavioral effects and, when delivered to the sleeping animal, did not result in waking from slow-wave sleep or from REM sleep. However, concurrent with stimulation were some EEG changes that might indicate some type of sub-behavioral changes in arousal level. One interpretation of these data is that stimulation of the nucleus raphé dorsalis in some manner blocks PGO activity as a secondary result of sub-behavioral arousal. However, these data are not inconsistent with the notion that nucleus raphé dorsalis neurons might exert tonic inhibitory control over PGO waves and possibly other classes of phasic events, except of course, during REM sleep.

NEUROCHEMICAL BASES OF THE PGO WAVE

Jouvet and his colleagues [39,40] have stressed that PGO waves depend upon catecholamines. One piece of evidence for this is that alpha-methyldopa, which is metabolized to the false transmitter alpha-methylnoradrenaline and displaces noradrenaline from stores, suppresses PGO spike activity. There is also some supporting evidence

from electrolytic and chemical lesions in the locus coeruleus. The most damaging evidence against this notion are the several studies [46,80] with alpha-methylparatyrosine, which selectively inhibits tyrosine hydroxylase and leads to a depletion of noradrenaline and dopamine. In these studies REM time increased and the total amount of PGO spikes were increased. High doses of this compound are nephrotoxic, however, and it is difficult to achieve total catecholamine depletion. In an effort to overcome this objection, Henriksen and Dement [27] administered alpha-methylparatyrosine to cats by intravenous drip in a dosage calculated to be supra-maximal and saw, as have several other investigators, a modest increase in the amount of slow-wave sleep, associated with a small decrease in the amount of wakefulness and, secondly, an increase in the amount of HEM sleep. Thus, they feel it is difficult to attribute PGO spike discharge to catecholaminergic neurons. Furthermore, Haefely et al. [20] have provided several lines of pharmacological evidence that catecholamines are actually involved in the inhibition of PGO spike activity.

Various investigators have pointed out that REM sleep is acutely susceptible to disruption by pharmacological intervention in the normal animal. Of course, this complicates the study of the neurochemical bases of any single component of the REM state. If a drug treatment blocks tonic muscle inhibition, produces nausea or discomfort, or raises the arousal level, it would block the occurrence of REM sleep and, therefore, secondarily could prevent the appearance of PGO waves. Dement's group has studied the PCPA-treated cat in which waking PGO waves can be studied without concern about the other REM components. They regard waking PGO waves as homologues to or displaced from REM sleep for several reasons, namely: (1) As the number of waking PGO waves increases after PCPA treatment, the number of PGO waves decreases per REM sleep epoch; (2) Following the discharge of a number of PGO waves during a REM sleep period in a PCPA-treated cat, waking waves only slowly reappear in the subsequent waking state; (3) both waking and REM sleep PGO waves frequently precede eye movements, while in the normal awake animal, lateral geniculate waves associated with eye movements always follow eye movements; (4) Finally, both the amplitude of waking and REM sleep PGO waves are recorded unattenuated from leads in visual cortex when the animal is placed in the dark, while the amplitude of cortical waves associated with waking eye movements in normal cats diminishes in the dark.

Using these findings, Dement's group waited until PGO waves appeared in the waking state as the result of pre-treatment with DL-PCPA (150 µg subcutaneously) and then administered the following presumptive receptor blockers: pimozide; phentolamine (alpha-adrenergic blocker) and propranalol (beta-adrenergic blocker). Their results were unequivocal in that none of the catecholamine receptor blockers had a significant effect on the rate of PGO wave discharge.

Dement et al. [28,32] also investigated the possible role of cholinergic mechanisms in PGO spike activity and, in order to study these, administered atropine to chronic PCPA-treated cats. They found that this compound totally blocked the occurrence of PGO waves in low doses and that this effect was partially reversed by subsequent injection of eserine. Evidence that the atropine effect was a direct one on the central nervous system derives from the fact that equivalent doses of atropine methylbromide, which does not cross the blood-brain barrier in significant quantities, had no effect on discharge rate of PGO waves. An observation of note in these studies was that the lowest dose of atropine which did suppress PGO spikes, did not produce the well-known EEG synchronization during the period of spike suppression.

Atropine's blocking of PGO activity was further substantiated in acute experiments. Unimplanted cats were pretreated with PCPA for five days and were then anesthetized and deep electrodes were positioned for recording PGO activity from both the pons and lateral geniculate nucleus. Once the level of anesthesia was adjusted for optimal PGO discharge, drugs were then administered and evaluated. Effects of PGO waves of atropine, atropine methylbromide, and eserine in these acute experiments were similar to findings in chronic animals regardless of where PGO activity was recorded. In view of the work of Gadea-Ciria and Jouvet [21], atropine-induced slow-waves and spindles in the neocortex might well be

assumed to have a secondary effect on PGO waves. However, since the atropine effect was obtained in both the lightly and deeply barbiturized animal, the non-specific effect of diminished arousal does not appear to be a likely explanation of the atropine suppression of PGO waves.

INTERACTIONS BETWEEN THE CATECHOLAMINERGIC SYSTEM AND THE SEROTONERGIC SYSTEM

The nature of interplay between these two major chemical systems in the brain is based on the following lines of experimental findings: (1) Lesions of the rostral third of the locus coeruleus or the ascending dorsal noradrenergic pathway at the level of the junction of the pons and midbrain in the cat induces a striking hypersomnia with increase in both slow-wave and HEM sleep, e.g., up to 300% in Jouvet's studies [39], for a period of 5-10 days. In these studies biochemical analysis showed a decrease of norepinephrine in the forebrain and midbrain and a significant increase of both tryptophan and 5-HIAA. These data indicate that damage to noradrenergic fibers arising from the anterior (rostral third) components of the locus coeruleus results in an increased serotonin turnover in the rostral raphé as demonstrated by an increase of 5-HIAA in the forebrain. In the nucleus raphé dorsalis region Loizou [51] has demonstrated many catecholamine terminals which are in a strategic position to affect, directly or indirectly, the activity of serotonin neurons. It is relevant to note that the increase of serotonin turnover is also accompanied by an increased level of tryptophan since this indicates that the uptake of tryptophan may play an influential role in the regulation of biosynthesis of brain serotonin; (2) In the rat destruction of catecholamine-containing neurons with 6-hydroxydopamine (injected intraventricularly or intracisternally) induces a highly significant increase in the synthesis of central serotonin as indicated by the increase of 5-HIAA and by an increase of labeled serotonin synthesized from labeled tryptophan injected intracisternally, whereas endogenous serotonin levels do not change. This phenomenon is observed as soon as 40 hrs after the injection of 6-hydroxydopamine and lasts as long as three weeks [45]. In the cat, however, direct injection of 6-OHDA into the central nervous system has been repeatedly found to lower brain serotonin and 5-HIAA levels [64,90,30]. Hence, important species differences in effects of this agent should be emphasized when attempting to interpret data across species; (3) In the cat the inhibition of catecholamine synthesis with alpha-methylparatyrosine induces, after approximately 8 hours, a significant increase of endogenous 5-HIAA and of labeled serotonin synthesized from labeled tryptophan in the lower brainstem (pons and medulla). Thus, there is a large body of evidence indicating interplay between the serotonergic and catecholaminergic systems in the brain.

The effects on the vigilance states obtained by surgical destruction of the catecholaminergic neurons have been partially verified by Jouvet [41,42] in the cat by the use of intraventricular 6-hydroxydopamine. These results can be summarized as follows: (1) About 15 days after intraventricular injection of 6-hydroxydopamine a decrease of catecholamine synthesis is observed in all structures of the brain as shown by a decrease of endogenous norepinephrine and dopamine and by a substantial decrease of labeled catecholamine (40-90%) and O-Methyl-^{3}H-catecholamine (25-90%) synthesized from exogenous ^{3}H-tyrosine. Under the same conditions, a significant decrease of the concentration of endogenous serotonin and 5-HIAA has been observed [41]; (2) During the period after 6-hydroxydopamine injection, when there are marked depletions in both serotonin and catecholamines, there is no significant alteration of the quantitative level of the cortical synchronization. However, cortical synchronization does, on occasion, appear during behavioral waking [42]. In these studies REM sleep is diminished according to the dose of 6-hydroxydopamine injected. The frequency of PGOs during REM sleep was shown to be permanently decreased by about 50% and this decrease affected mostly the bursts of PGO. However, in chlorimipramine-pretreated cats 6-hydroxydopamine produces several interesting findings: (a) No alteration of cerebral indoleamines while cerebral catecholamines are decreased and (b) significant (50%) and permanent increases of cortical synchronization with REM sleep returning to normal levels after about six days. but

decreasing thereafter; and (c) no alteration of the rate of PGO activity during REM sleep. Taken together these results all suggest that cortical synchronizing and desynchronizing mechanisms which act during slow-wave sleep and waking are under the regulation of antagonistic serotonin and catecholaminergic systems.

REM sleep appears to involve, according to Jouvet and others, some serotonin "priming" mechanisms which appear to be located in the pontine group of nuclei of the raphé system, i.e., the nucleus raphé pontis and nucleus raphé magnus, whereas its so-called "executive" mechanisms may partly depend upon catecholaminergic, and possibly cholinergic, neurons located in the nuclear complex of the nucleus locus coeruleus and subcoeruleus. In this regard, the ponto-geniculo-occipital (PGO) activity, which is highly characteristic of REM sleep, has been selectively suppressed in the lateral geniculate nucleus by extensive lesions of the locus subcoeruleus or by lesions of a pathway originating from this region, ascending in the mesencephalon, and coursing in the supraoptic decussation [49].

Some complex interactions exist between the serotonergic systems responsible for sleep and the catecholaminergic system responsible for waking, while there appears to be agonistic interaction between the serotonin and the noradrenergic mechanisms which are involved in REM sleep. The intimate nature of these regulations is not yet understood but they may explain the interactions which exist between the increased waking and the following rebounds of both slow-wave sleep and REM. The increased waking which follows the destruction of the anterior nuclei raphé is most probably mediated by catecholaminergic mechanisms, as shown by the following experiments carried out by Jouvet and his group: An injection of 200 mg/kg of alpha-methylparatyrosine was given to cats with raphé destruction at a time when behavioral and EEG waking was almost permanent (2-6 days after the lesion). The animals then showed cessation of running movements, miosis appeared, and there was an almost continuous cortical synchronization. This lasted for approximately 24 hrs. after which time there was a rapid return to behavioral and EEG insomnia. This experiment provides some neuropharmacological evidence that the almost permanent arousal which follows the destruction of the rostral group of the serotonin-containing neurons of the raphé system might be related to the increased turnover in central catecholaminergic neurons. From these results it is possible to conclude that, under normal conditions, serotonin neurons might exert some tonic inhibitory action, directly or indirectly, upon some catecholamine-containing neurons at the onset of sleep. The nature of the interconnecting circuitries that mediate these interactions is now under intensive investigation.

The hypothesis that the serotonin-containing neurons play a role in the "priming" of REM is founded on the following facts: (1) It appears that there must exist a critical minimum of slow-wave sleep in order for REM to appear (after either sub-total lesion of the raphé system or inhibition of the serotonin synthesis with PCPA). Indeed, Jouvet reports that a statistical correlation exists between the amount of slow-wave sleep and REM sleep. For example, in the cat the percent of REM sleep equals percent of (slow-wave sleep - 16) / 3.2. This holds true for cats subjected to lesions of the raphé system (with decrease of serotonin turnover) and for hypersomniac cats subjected to lesions of the dorsal noradrenergic bundle (with increase of serotonin turnover). Thus, it appears that a minimum of 16% of daily slow-wave sleep, which is roughly one-third of the normal amount, is necessary to "actuate" REM sleep mechanisms. Since there is some relationship between the amount of slow-wave sleep and serotonin turnover, this would suggest that any important decrease of serotonin turnover, induced either by a lesion or drug, would decrease or suppress REM, although other interpretations are possible, such as the suppression of deaminated metabolites of serotonin (5-hydroxyacetaldehyde or 5-hydroxytryptophol) which may play some physiological role [74]. These findings might explain why the inhibitors of monoamine oxidase, which decrease the serotonin turnover, are very potent suppressors of REM sleep.

Another possible mechanism for the "priming" or actuating of REM sleep by the prior occurrence of slow-wave sleep involves the possible role of growth hormone secretion. Since we [82] have

recently reported that injection of growth hormone into cats increases the occurrence of REM sleep, it may be that the prior release of growth hormone during slow-wave sleep is the reason why REM always follows slow-wave sleep. However, the large release of growth hormone during slow-wave sleep has so far only been reported in primates [84].

SOME REMARKS ON THE SEROTONIN-SLEEP HYPOTHESIS

The cellular mechanisms by which either serotonin cells of the raphé complex or neurons of the locus coeruleus are activated are not known, although there is now ample evidence that noradrenergic fibers from the locus coeruleus synapse on serotonergic cells of the nucleus raphé dorsalis and are in ideal position to regulate activity in this part of the raphé complex. As noted we [57] have shown that electrical stimulation of the locus coeruleus inhibits cell firing in the nucleus raphé dorsalis. What are now needed are studies of the correlations between cell firing in specific brainstem areas, such as the raphé and locus coeruleus, and amine release. It might be assumed that cells of the raphé nuclei would discharge more rapidly in slow-wave sleep and those of the locus coeruleus more rapidly in REM sleep. Sheu et al. [79] showed, on the contrary, that cells of the nucleus raphé medianus and nucleus raphé magnus fire more slowly in slow-wave sleep than in waking or REM sleep. McGinty et al. [55] likewise showed that cells of the nucleus raphé dorsalis fire much slower in slow-wave sleep and REM sleep as compared to waking. It has been stressed by McGinty [54] that the slowing of raphé unit firing during sleep contradicts current theories suggesting a facilitating role for serotonin in sleep and these studies do indeed constitute gathering evidence against a direct serotonin-raphé- slow-wave sleep hypothesis. Hishikawa et al. [29] showed that with imipramine there is a great increase in slow-wave sleep in the cat but during this slow-wave sleep the raphé is not showing an increase in firing at the unit level as indicated by the studies of Aghajanian et al. [1]. On the other hand, Chu and Bloom [10] did show that norepinephrine-containing cells of the locus coeruleus fire fastest in REM sleep, thus supporting the view that increased activity in the locus coeruleus is associated with an increase in REM sleep. Some indirect evidence in favor of the raphé-serotonin-slow-wave sleep theory comes largely from pharmacological experiments. The most convincing of these shows that when tryptophan hydroxylase is blocked with PCPA, serotonin synthesis decreases, levels of serotonin in the brain are reduced, and insomnia of several days duration ensues. Circumventing this block with 5-HTP restores both serotonin levels and sleep. However, when the depletion experiments are performed chronically, animals recover their sleep within several days even though serotonin is maintained at extremely low levels by repeated injections of PCPA. This latter finding does place in some doubt at least the apparent uniqueness of the role of serotonin in sleep.

In vivo studies of the turnover of the biogenic amines in relation to natural sleep and waking states are also greatly needed. Presently it is still only possible to conclude that the biogenic amines appear to co-vary with experimentally-induced changes in sleep and waking and vice versa. Normal functioning of their metabolic and neuronal pathways would then appear to be necessary for the occurrence of normal sleep., However, the nuclei, chemical pathways and circuits cannot yet be justifiably identified as a "sleep system" or "sleep center," nor can the amines themselves be tagged as hypnogens or sleep substances. They may serve as actuator agents but their role in maintenance of a particular vigilance state is highly questionable. The fact that the amine-containing neurons lie in brainstem areas that are known to be important for normal sleep makes the cellular approach in relation to amine release the one that might eventually prove to be the most informative about mechanisms. The idea of a "sleep substance" long antedates the demonstration of neurohumoral synaptic transmission in the central nervous system. Thus, Piéron [68] performed experiments to test his hypothesis that a "hypnotoxin" gradually accumulated during waking and then, reaching a critical level, depresses the brain, resulting in sleep. In these experiments the cerebrospinal fluid of sleep-deprived dogs was found sometimes to produce somnolence when

injected into the ventricles of normal recipients. However, various unphysiological factors were later found to be involved in this approach by Schnedorf and Ivy [77]. Thus, some recipient animals were found to become feverish, possibly owing to contamination of the transferred cerebrospinal fluid. Some of the technical limitations have been overcome in the recent studies by Pappenheimer and his group [65] who have been able to obtain large amounts of cerebrospinal fluid from goats and re-inject extracts of these into smaller animals under completely sterile and hydrodynamically controlled conditions. However, in most of their reported work they have used "inactivity" of the recipients as a measure of sleep. So far, the work of Fencl et al. [17], as well. as that of Monnier et al. [58,78] dealing with humoral factors presumably responsible for the induction of sleep, have not specifically isolated the substance although the search still goes on.

Regardless, no conclusive evidence has been gathered up to this time to consider any of the biogenic amines themselves, or even their primary metabolites, as the sleep-inducing substance. An extreme example of this attempt was the proposal by Koella [47] to re-label serotonin as "somnotonin", with the observation that serotonin is the (or one component of) "hypnotoxin" responsible for sleep. In our studies we have shown conclusively that the tryptophols (metabolites of serotonin) are not themselves sleep-inducing agents. Indeed, they actually produce increased waking in the cat (Fig 4).

FIGURE 4

EFFECTS OF TRYPTOPHOL ON SLEEP AND WAKING IN CATS

Mean (+S.D.) percent of total time

			TRYPTOPHOL (mg/kg)	
	BASELINE n=13	**TWEEN-80** n=4	100-200 n=6	400 n=4
WAKING	38 (±5)	26 (±19)	46 (±15)	71 (±10)
SWS	50 (±6)	63 (±13)	49 (±11)	29 (±11)
REM SLEEP	13 (±3)	12 (±4)	5 (±4)	1 (±1)
TOTAL SLEEP	63 (±6)	74 (±19)	54 (±19)	30 (±10)
REM / T.S.	20 (±4)	14 (±8)	8 (±3)	2 (±2)

Figure 4. Effect of intra-peritoneal administered (suspended in *Tween-80) tryptophol on the vigilance states in the cat. Note a dose-related decrease of both slow-wave sleep and REM sleep following tryptophol administration and a corresponding increase* in *waking behavior.*

How can we now view the serotonin-sleep hypothesis if brain serotonin can be reduced by up to 90% while producing sleep losses only in the range of 0 to 35%? In the cat, Jouvet [37] reported that a single injection of PCPA produces an almost total insomnia lasting from approximately the 30th to 60th hour following injection. Agreed that this appears to be a support for the serotonin-sleep hypothesis, two considerations suggest a need for cautious interpretation. Both Jouvet [37] and Dement [15] have noted that during the PCPA-induced insomnia there is a striking and almost continuous appearance of the PGO waves which normally occur mostly within REM sleep. If, then, this is not a "normal" state of wakefulness, it does raise the issue of whether serotonin depletion can account for physiological wakefulness. Serotonin might very well play a determinant role in sleep, but since we are also probing into the origin of normal wakefulness, we will very likely have to consider its genesis as more "actively" derived and not simply due to a lowering of brain serotonin at some critical site in the brain. Secondly, Dement [15] has studied cats who were chronically administered PCPA and insomnia developed in these animals in a manner similar to that described by Jouvet. By the end of the first week of drug administration, however, sleep was returning to approximately 70% of baseline values, although brain serotonin was almost totally depleted at this point in time. A parallel phenomenon was also seen in the rat. Further, data of Mouret et al. [60] show that the recovery of brain serotonin after PCPA administration lags considerably behind the recovery of sleep, e.g., by the 10th day following PCPA administration slow-wave sleep had returned to approximately 85% of baseline values, whereas brain serotonin had only reached 55% of baseline values.

Several possible counter-arguments to the lack of temporal correspondence between serotonin recovery and sleep recovery are possible. It might be that sleep recovery precedes serotonin recovery because of the fatigue of waking mechanisms during the period of insomnia [72]. But if we assume additional determinants of sleep, such as a "fatiguing" of wakefulness, which itself is not dependent on serotonin, in effect this argument diminishes the importance of serotonergic-induced sleep mechanisms.

It has also been argued that the actual occurrence of sleep is determined by two largely independent factors, a sleep need and a sleep mechanism. According to this view, serotonin depletion might interfere with the sleep mechanism, but the accumulation of the need during the period of the insomnia might drive the impaired sleep mechanism to perform to its limit. As noted by Rechtschaffen et al. [72], this is not an unreasonable position, but, again, it postulates a non-serotonergic sleep determinant, which, in turn, diminishes the role of serotonergic factors and brings into question some major foundations of the monoaminergic theory of the vigilance states.

With respect to other species, Weitzman et al. [88] have shown modest sleep decrements with moderate PCPA-induced serotonin decrements in monkeys and this data appears to be consistent with the serotonin-sleep hypothesis. In humans Wyatt et al. [89] found that PCPA produced a significant decrease in the amount of REM sleep in four carcinoid patients. In three of the patients there was no change in the amount of non-REM sleep while in the fourth there was a small but significant increase in non-REM sleep. Such negative findings have generally not been taken very seriously, presumably because massive brain serotonin decrements cannot be produced in humans with oral PCPA. Yet there is considerable evidence that sleep and wakefulness vary spontaneously within each day without overwhelming oscillations in brain serotronin. Why, then, are such tremendous serotonin changes needed in order to produce effects on sleep? It could be that serotonin is acting at a specific receptor site in the brain and that significant elevations of serotonin are needed to perfuse the. brain adequately and get at the target zone in critical amounts sufficient to "trigger" the necessary mechanisms for sleep induction.

Along these same lines, the work of Hartmann [22] shows that neither a tryptophan-free diet nor a tryptophan-rich diet significantly affects total sleep in the rat. The failure of a tryptophan-free diet, which decreases brain serotonin by some 40%, or a tryp-

tophan-rich diet to affect significantly total sleep time in rats appears to be contrary to the serotonin-sleep hypothesis. Such evidence should not be neglected simply because the brain serotonin changes are not as large as those produced by serotonin inhibitors and precursors. Hartmann's experiments do have the advantage at least of mimicking physiological routes of serotonin change, and it would presumably be by such routes that sleep and wakefulness might be normally regulated by serotonin.

Radulovacki [71] has also assembled some evidence that can be considered contrary to the serotonin-sleep hypothesis. He implanted cannula into the cisterna magna of cats so as to be able to draw cerebrospinal fluid repeatedly without disturbing a sleeping or waking cat. He failed to find a correlation between the concentration of 5-HIAA in the cisternal cerebrospinal fluid as a function of sleep and waking states. In many samplings from cats during wakefulness, slow-wave sleep and REM sleep he found no significant differences in mean values of 5-HIAA concentration.

On the whole there appears to be no suitable assembly of hard evidence which makes the acceptance of the serotonin-sleep hypothesis obligatory. For most types of evidence that have been offered in its favor there is either contradictory evidence or certainly reasonable alternative interpretations. Some of these alternative interpretations result from problems intrinsic to the determination of the physiological role of any single chemical agent in regulating a total or global behavior. Studies which correlate spontaneous changes in brain chemistry with spontaneous changes in behavior have the advantage of following physiological variations, but as to questions of cause and effect, these latter must be approached by a variety of experimental manipulations. In experimentally manipulating the biochemical events of the brain we can never be certain that we are mimicking the natural course of events. At present there simply is not a wholly adequate accumulation of supporting evidence for the serotonin-sleep hypothesis and now there is considerable evidence being gathered to the contrary. However, this evidence does not rule out serotonin as playing some role in sleep mechanisms or that norepinephrine-serotonin interactions are not critical parts of the chemical oscillations involved in transitions from one vigilance state to another. However, this raises the question as to exactly what is meant by the monoamine theory of sleep-waking behavior. It does not imply a simple relationship such as how saturated the brain is with serotonin or norepinephrine and how much of each type of sleep is present. The evidence is quite clearly against such simple direct relations. Examining the PCPA data on rats, for example, how can we explain the fact of moderate sleep decrements with up to 90% depletion in brain serotonin, while Scheving et al. [76] report that brain serotonin levels fluctuate spontaneously only an approximately 18% range during any 24 hour period? Quite clearly, the normal rat would not likely require a 90% depletion of brain serotonin to maintain wakefulness.

Something much more complex than gross serotonin or norepinephrine levels, or, for that matter, gross serotonin or noreinephrine turnover, is involved in controlling sleep and the oscillations between the states of vigilance. Some aspect of serotonin or norepinephrine activity is likely to be varying spontaneously at some specific receptor site in the brain, probably in interaction with other chemical systems, to affect neuronal discharge patterns which, in turn, contribute to specific aspects of sleep-waking control. As Rechtschaffen et al. [72] and others have pointed out, by varying the whole brain chemistry, we are testing only the rather crude hypothesis, i.e., one amine effect on one sleep state.

Hopefully, by careful use of more specific chemical lesioning agents placed in particular chemoanatomical pathways and by studying local variations in brain amine turnovers in relation to the vigilance states we may move nearer to unraveling the role of the monoamines in sleep and other behaviors. At least it appears we are well beyond the "one amine--one behavior" stage of reasoning. Neuron firing in relation to each state of vigilance and in relation to amine release is now beginning to provide valuable insights at the cellular level. Studies of interactions between chemical systems in the brain appears to be one of the most profitable approaches for future studies. Once enough is known about the chemical

scaffolding in the brain the proper experiments can be designed to test the interactions among these systems. Massive manipulation of brain chemistry by pharamacological or lesioning methods has so far provided us with some general and informative insights into the role of monoamines in sleep-waking activity but these methods have not yet identified the chemical agent(s) responsible for actuating or maintaining each state of vigilance or shed much light on how the several chemical systems interact to generate behavior. Obviously, the uniqueness of any chemical agent in behavior is still very much in doubt. At the present time we can probably agree that the biogenic amines serotonin and norepinephrine appear to be involved in some aspects of generation and maintenance of the vigilance continuum. However, by no means can we, at present, accept the view that these biogenic amines are themselves physiological sleep-inducing substances.

Jouvet [44] has recently stressed that we should no longer ask the question as to whether serotonin neurons play a role in sleep mechanisms but rather we should ask how do serotonin neurons regulate sleep? Although we cannot at the present time state, with any degree of certainty, how the monoamines regulate sleep we have at least taken some stock of the field and brought several lines of research and their interpretation under critical scrutiny. We have reviewed some of the various evidence for and against monoamine regulation of the states of vigilance and hinted at lines of future investigation. It is clear that there is still a long way to go before we are on solid ground in relating chemistry of the brain to behavior.

ACKNOWLEDGMENT

Supported by National Science Foundation grant GB 43529x and by grants MH 10625 and MH 02211, National Institute of Mental Health.

REFERENCES

1. Aghajanian, G.K., B.S. Bunney and M.J. Kuhar. Use of single unit recording in correlating transmitter turnover with impulse flow in monoamine neurons. IN: New Concepts in Neurotransmitter Regulation, A.J. Mandell, Ed., New York, Plenum Press, 1973, pp 115-145.

2. Aghajanian, G.K., J.A. Rosecrans and M.H. Sheard. Serotonin: Release in the forebrain by stimulation of midbrain raphé. Science 156: 402-403, 1967.

3. Andén, N.E., H. Corrodi, K. Fuxe and T. Hokfelt. Increased impulse flow in bulbospinal NA neurons by catecholamine receptor blocking agents. Europ. J. Pharmacol. 2: 59-64, 1967.

4. Andén, N.E., H. Corrodi, K. Fuxe and U. Ungerstedt. Importance of nervous impulse flow for the neuroleptic induced increase in amine turnover in central dopamine neurons. Europ. J. Pharmacol. 15: 193-199, 1971.

5. Andén N.E., A. Dahlström, K. Fuxe, K. Larsson, L. Olson and U. Ungerstedt. Ascending monoamine neurons to the telencephalon and diencephalon. Acta Physiol. Scandf. 67: 313-326, 1966.

6. Baumgarten, H.G., A. Bögrklund, L. Lachenmayer, A. Nobin and U. Stenevi. Long-lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. Acta Physiol. Scand. Suppl. 373: 1-15, 1971.

7. Benda, P. and A. Perlés. Étude expérimentale de l'abaissement de la vigilance par la gamma-butyrolactone. Comp. Rend. Soc. Biol 251: 1312-1313, 1960.

8. Bobillier, P., J.L. Proment, S. Seguin and M. Jouvet. Effets de la P. Chlorophenylalanine et du 5-hydroxy-

tryptophane sur le sommeil et le métabolisme central des monoamines et des protéines chez le chat. Biochemical Pharmacology 1974 (in press).

9. Carlsson, A. and M. Lindqvist. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normethanephrine in mouse brain. Acta Pharmacol. Toxicol. 20: 140-144, 1963.

10. Chu, N. and F.E. Bloom. Norepinephrine-containing neurons: Changes in spontaneous discharge patterns during sleeping and waking. Science 179: 908-910, 1973.

11. Corrodi, H., K. Fuxe, P. Lidbrink and L. Olson. Minor tranquilizers, stress and central catecholamine neurons. Brain Res. 29: 1-16, 1971.

12. A. Dahlström and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. Acta Physiol Scand. 62: 1-55, 1964.

13. Dement, W.C., S. Henriksen, B. Jacobs and M.M. Mitler. Biogenic Amines - Phasic events and behavior. IN: Pharmacology and the Future of Man. Basel, Switzerland, Karger, 1973, pp 74-89.

14. Dement, W.C., M.M. Mitler and S. Henriksen. Sleep changes during chronic administration of parachlorophenylalanine. Rev. Can. Biol. 31: 239-246, 1972.

15. Dement, W.C., V. Zarcone, J. Ferguson, H. Cohen, T. Pivik and J. Barchas. Some parallel findings in schizophrenic patients and serotonin depleted cats. IN: Schizophrenia: Current Concerts and Research D.V. Siva Sankar, Ed., Hicksville, N.Y., PJD Publications LTD., 1969, pp 775-811.

16. Descarries, L. and G. Saucier. Disappearance of the locus coeruleus in the rat after intraventricular 6-hydroxydopamine. Brain Res. 37: 310-316, 1972.

17. Fencl, V., G. Koski and J.R. Pappenheimer. Factors in cerebrospinal fluid from goats that affect sleep and activity in rats. J. Physiol. 216: 565-589.

18. Fuxe, K., T. Hökfelt and U. Ungerstedt. Morphological and functional aspects of central monoamine neurons. Int. Rev. Neurobiology 13: 93-126, 1970.

19. Fuxe, K. and U. Ungerstedt. Histochemical biochemical and functional studies on central monoamine neurons after acute and chronic amphetamine administration. IN: Amphetamine and Related Compounds. E. Costa and S. Garattini. Eds.. New York, Raven Press, 1970, pp 257-288.

20. Haefely, W., M. Jalre and M.A. Monachon. NE-neurons and phasic sleep. Biochemical Pharmacology Supp., 638-640, 1974.

21. Gadea-Ciria, E. and M. Jouvet. Corticofugal control of pontine PGO activity in the cat. Psychophysiology 9: 129, 1972.

22. Hartmann, E. The effect of LSD and L-tryptophane on the sleep-dream cycle in the rat. Psychonomic Science 8: 295-296, 1967.

23. Hartment, E. The biochemistry and pharmacology of the D-state (dreaming sleep). Experimental Medicine and Surgery 27: 105-120, 1969.

24. Hartmann, E. The D-state and norepinephrine-dependent systems. IN: Sleep and Dreaming, E. Hartmann, Ed., Boston, Little Brow; and Co., 1970, PP 308-328.

25. Harner, R.N. and R.M. Dorman. A role for dopamine in control of sleep and wakefulness. Trans. Amer. Neurological Assn. 95: 252-254, 1970.

26. Henley, K. and A.R. Morrison. A re-evaluation of the effects of lesions of the pontine tegmentum and locus coeruleus on phenomena of paradoxical sleep in the cat. Acta Neurobiol. Exp. 34: 215-232, 1974.

27. Henriksen, S. and W. Dement. Effects of chronic intravenous administration of 1-alpha-methylparatyrosine on sleep in the cat: A preliminary investigation. IN: Sleep Research Vol. 1, M. Chase, W. Stern, and P. Walter, Eds. Brain Information Sevice/Brain Research Institute, U.C.L.A., Los Angeles, 1972, p 55.

28. Henriksen, S.J., B.L. Jacobs and W.C. Dement. Dependence of REM sleep PGO waves on cholinergic mechanisms. Brain Res. 48: 412-416.

29. Hishikawa, Y., K. Nakai, I. Hidenobu and Z. Kaneko. The effect of imipramine, desmethylimipramine and chlorpromazine on the sleep-wakefulness cycle of the cat. Electroenceph. clin. Neurophysicol. 19: 518-521, 1965.

30. Howard, J.L. and G.R. Breese. Physiological and behavioral effects of centrally-administered 6-hydroxydopamine in cats. Pharmacol. Biochem. and Behavo. 2: 651-661, 1974.

31. Jacobs, B.L., R. Asher and W.C. Dement. Electrophysiological and behavioral effects of electrical stimulation of the raphé nuclei in cats. Physiol. and Behavior 11: 489-495, 1973.

32. Jacobs, B.L., S.J. Henriksen and W.C. Dement. Neurochemical bases of the PGO wave. Brain Res. 48: 406-411, 1972.

33. Jonsson, G., K. Fuxe and J. Daly. Intracerebral injections of 5,6-dihydroxytryptamine (5,6-HT). Evidence for selective degeneration central 5-hydroxytryptamine (5-HT) neurons. Acta Pharmacol. Toxicol. 31: Suppl. 1, 24, 1972.

34. Jones, B.E., P. Bobillier and M. Jouvet. Effets de la destruction des neurones contenant des catécholamines du mésencéphale sur le cycle veille-sommeils du chat. Compt. Rend. Soc. Biol. 163: 176-180, 1969.

35. Jones, B.E., P. Bobillier, C. Pin and M. Jouvet. The effect of lesions of catecholamine-containing neurons upon monoamine content of the brain and EEG and behavioral waking in the cat. Brain Res. 58: 157-177, 1973.

36. Jouvet, M. Mechanisms of the states of sleep: A neuropharmacological approach. IN: Sleep and Altered States of Consciousness. S. Kety, E. Evarts and H. Williams, Eds. Baltimore, The Williams and Wilkins co., Vol. 45, 1967, pp 86-126.

37. Jouvet, M. Biogenic amines and the states of sleep. Science 163: 32-41, 1969.

38. Jouvet, M. Some monoaminergic mechanisms controlling sleep and waking. IN: Brain and Human Behavior, A.G. Karczmar and J.C. Eccles, Eds., New York, Springer Verlag, 1972, PP 131-161.

39. Jouvet, M. The role of monoamines and acetylcholine-containing neurons in the regulation of the sleep-waking cycle. Ergebnisse der Physiologie 64: 166-307, 1972.

40. Jouvet, M. Serotonin and sleep in the cat. IN: Serotonin and Behavior J. Barchas and E. Usdin, Eds., New York, Academic Press, 1973, pp 385-400.

41. Jouvet, M. Monoaminergic regulation of the sleep-waking cycle. IN: Pharmacology and the Future of Man. Basel, Switzerland, Karger, 1973, pp. 103-107.

42. Jouvet, M. The role of monoaminergic neurons in the regulation and function of sleep. IN: Basic Sleep Mechanisms. O. Petre-Quadens and D.J. Schlag, Eds., New York, Academic Press, 1974, pp 207-236.

43. Jouvet, M. and J.F. Pujol. Les médiateurs chimiques--Leur role dans la physiopathologie de la motricite de la vigilance et du comportement. Etude neurophysiologique et biochimique. Revue Neurologique 127: 115-138, 1972.

44. Jouvet, M. and J.F. Pujol. Effects of Central alterations of serotoninergic neurons upon the sleep-waking cycle. IN: Advances in Biochemical Psychopharmacology. E. Costa, G.L. Gessa and M. Sandler Eds. Vol. 11 New York, Raven Press, 1974, pp 199-209.

45. Juge, A., F. Sordet, M. Jouvet and J. Pujol. Modification du métabolisme de la sérontonine cérébrale aprés 6-hydroxydopamine chez le rat. Comp. Rnd. Acad. Sci. 274: 3266-3268, 1972.

46. King, C.D. and R.E. Jewett. The effects of alpha-methyltyrosine on sleep and brain norepinephrine in cats. J. Pharmacol. Exp. Ther. 177: 188-195, 1971.

47. Koella, W.P. Discussion of insomnia and decrease of cerebral 5-hydroxytryptamine after destruction of the raphé system in the cat. IN: Advances in Pharmacology S. Garattini and P.A. Shore, Eds., Vol.. 6, Part B, New York, Academic Press, 1968, pp 280-282.

48. Kostowski, W., E. Giacalone, S. Farattini and L. Valzelli. Electrical stimulation of midbrain raphé: Biochemical behavioral and bioelectrical

effects. European J. Pharmacol. 7: 170-175, 1969.

49. Laurent, J.P., R. Cespuglio and M. Jouvet. D&limitation des voies ascendantes responsables de. l'activaté ponto-géniculo-occipitale chez le chat. Experientia 28: 1174-1175, 1972.

50. Lidbrink, P., H. Corrodi, K. Fuxe and L. Olson. On the role of central monoamine neurons in sleep mechanisms. IN: Pharmacology and the Future of Man. Basel, Switzerland, Karger, 1973, pp 90-102.

51. Loizou, L.A. Projects of the nucleus locus coeruleus in the albino rat. Brain Res. 15: 563-566, 1969.

52. Maeda, T., C. Pin, D. Salvert, M. Ligier and M. Jouvet. Les neurones contenant des catécholamines du tegmentum pontique et leurs voies de projection chez le chat. Brain Res. 57: 119-152, 1973.

53. Mandell, A.J. and C.E. Spooner. Psychochemical research studies in man. Science 162: 1442-1453, 1968.

54. McGinty, D.J. Neurochemically defined neurons: Behavioral correlates of unit activity of serotonin containing cells. IN: Brain Unuit Activity During Behavior. M.I. Phillips, Ed., Springfield, Chas. C. Thomas, 1973, pp 244-267.

55. McGinty, D.J., R.M. Harper and M.K. Fairbanks. 5-HT-containing neurons: Unit activity in behaving cats. IN: Serotonin and Behavior, J. Barchas and E. Usdin, Eds., New York, Academic Press, 1973, pp 267-279.

56. Morgane, P.J. and W.C. Stern. Chemical anatomy of brain circuits in relation to sleep and wakefulness. IN: Advances in Sleep Research, E. Wietzman, Ed., New York, Spectrum Publications, 1974, pp 1-131.

57. Morgane. P.J., W.C. Stern and E. Berman. Inhibition of unit activity in the anterior raphé by stimulation of the locus coeruleus. Anatomical Record 178: 421, 1974.

58. Monnier, M. and E. Schoenenberger. Neuro-humoral coding of sleep by the physiological sleep factor delta. IN: Neurohumoral Coding of Brain Function. R.D. Myers and R. Drucker-Colin, Eds., New York, Plenum Press, 1974, pp 207-232.

59. Moruzzi, G. The sleep-waking cycle. Ergebnisse der Physiologie 64: 1-165, 1972.

60. Mouret, J., P. Bobillier and M. Jouvet. Insomnia following parachlorophenylalanin in the rat. European J. Pharmacol. 5: 17-22, 1968.

61. Nyback, H. Effect of brain lesions and chlorpromazine on accumulation and disappearance of catecholamines formed in vivo from ^{14}C-tyrosine. Acta Physiol. Scand. 84: 54-64, 1972.

62. Nyback, H. and G. Sedvall. Further studies on the accumulation and disappearance of catecholamines formed from tyrosine- ^{14}C in mouse brain. Effect of some phenothiazine analogues. European J. Pharmacol. 10: 193-205 , 1970.

63. Olson, L. and K. Fuxe. On the projections from the locus coeruleus noradrenaline neurons: The cerebellar innervation. Brain Res. 28: 165-171, 1971.

64. Panksepp, J., J.E. Jalowiec, P.J. Morgane, A.J. Zolovick and W.C. Stern. Noradrenergic pathways and sleep-waking states in cats. Experimental Neurology 41: 233-245, 1973.

65. Pappenheimer, J.R., T.B. Miller and C.A. Goodrich. Sleep-promoting effects of cerebrospinal fluid from sleep-deprived goats. Proc. Nat. Acad. Sci. 58: 513-517, 1967.

66. Petitjean, F. and M. Jouvet. Hypersomnie et augmentation de l'acide 5 hydroxy-indolacétique cérébral par lésion isthmique chez le chat. Comp. Rnd. Acad. Soc. 164: 2288-2293, 1970.

67. Petitjean, F., R. Laguzzi, F. Sordet, M. Jouvet and J.F. Pujol. Effets de l'injection intraventriculaire de 6-hydroxydopamine. I. Sur les monoamines cérébrales du chat. Brain Res. 48: 281-293, 1972.

68. Piéron, H. Le Probleme Physiologique du Sommeil. Paris, Masson, 1913.

69. Poirier, L.J., P. Langelier, A. Roberge, R. Boncher and A. Kitsikis.

Non-specific histopathological changes induced by the intracerebral injection of 6-hydroxy-dopamine (6-OH-DA). J. Neurol. Sci. 16: 401-416, 1972.

70. Polc, P. and M. Monnier. An activating mechanism in the ponto-bulbar raphé system of the rabbit. Brain Res. 22: 47-61, 1970.

71. Radulovacki, M. 5-Hydroxyindole-acetic acid in cerebrospinal fluid: measurements in wakefulness, slow-wave and paradoxical sleep. Brain Res. 50: 484-488, 1973.

72. Rechschaffen, A., R.A. Lovell, D.X. Freedman, W.E. Whitehead and M. Aldrich. The effect of parachloro-phenylalanine on sleep in the rat: Some implications for the serotonin-sleep hypothesis. IN: Serotonin and Behavior, J. Barchas and E. Usdin, Eds., New York, Academic Press, 1973, pp 401-418.

73. Roth, R.H. and Suhr, Y. The mechanism of the gamma-hydroxy-butyrate-induced increase in brain dopamine and its relationship to 'sleep'. Biochem. Pharmacol. 19: 3001-3012, 1970.

74. Sabelli, H., W. Giardina, S. Alivisatos, P. Seth and F. Ungar. Indoleacetaldehydes: Serotonin-like effects on the central nervous system. Nature (London) 223: 73-74, 1969.

75. Saner, A., L. Pieri, J. Moran, M. DaPrada and A. Pletscher. Decrease of dopamine and 5-hydroxytryptamine after intracerebral application of 5,6-dihydroxytryptamine. Brain Res. 76: 109-117, 1974.

76. Scheving, L., W. Harrison, P. Gordon and J. Pauly. Daily fluctuation (circadian and ultradian) in biogenic amines of the rat brain. American J. Physiology 214: 166-173, 1968.

77. Schnedorf, J.G. and A.C. Ivy. An examination of the hypnotoxin theory of sleep. American J. Physiology 125: 491-505, 1939.

78. Schoenenberger, G.A., L.B. Cueni, A.M. Hatt and M. Monnier. Isolation and physical-chemical characterization of a humoral, sleep inducing substance in rabbits (factor 'delta'). Experi-entia 28: 919-922, 1972.

79. Sheu, Y., J.P. Nelson and F.E. Bloom. Discharge patterns of Cat raphé neurons during sleep and waking. Brain Res. 73: 263-276, 1974.

80. Stern, W.C. and P.J. Morgane. Effects of alpha-methyltyrosine on REM sleep and brain amine levels in the cat. Biological Psychiatry 6: 301-306, 1973.

81. Stern, W.C. and P.J. Morgane. Effects of reserpine on sleep and brain biogenic amine levels in the cat. Psychopharmacologia (Berlin) 28: 274-286, 1973.

82. Stern. W.C. and P.J. Morgane. Theoretical view of REM sleep function: Maintenance of catecholamine systems in the central nervous system. Behav. Biol. 11: 1-32, 1974.

83. Stern, W.C., J.E. Jalowiec, H. Shabshelowitz and P.J. Morgane. Effects of growth hormone on sleep-waking patterns in cats. Hormones and Behavior 6: 189-196, 1975.

84. Takahashi, Y., D. Kipnis and W. Daughaday. Growth hormone secretion during sleep. J. Clin. Invest. 47: 2079-2090, 1968.

85. Ungerstedt, U. 6-Hydroxy-dopamine induced degeneration of central mono-amine neurons. European J. of Pharma-cology 5: 107-110, 1968.

86. Ungerstedt, U. Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. Acta Physiol. Scand. Suppl. 367: 95-122, 1971.

87. Ungerstedt, U. Histochemical studies on the effect of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine-contain-ing neurons in the CNS. IN: 6-Hydroxydopamine and Catecholamine Neurons. T. Malmfors and H. Thoenen Eds., Amsterdam, North-Holland Publish: ing Co., 1971, pp 1-48.

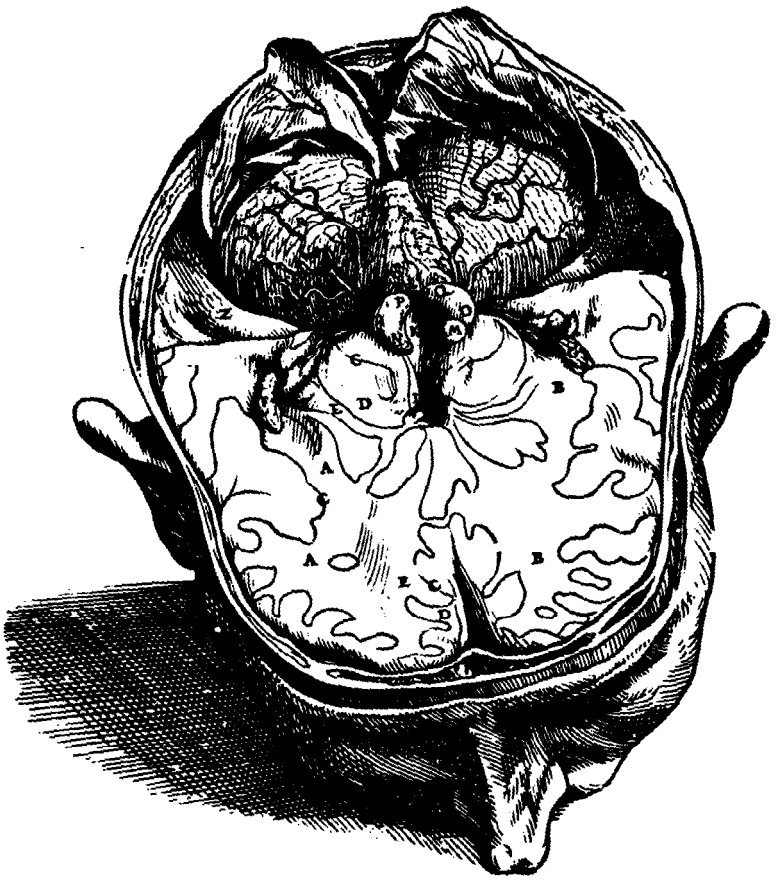
88. Weitzman, E., M. Rapport. P. McGregor and J. Jacoby. Sleep patterns of the monkey and brain serotonin concentration: Effect of p-chloro-phenyl-alanine. Science 160: 1361-1363, 1968.

89. Wyatt, R., A. Sjoerdsma and F. Snyder. Effects of parachlorophenylalanine on sleep in man. EEG. Clin. Neurophysiology 27: 529-532, 1969.

90. Zolovick, A.J., W.C. Stern, J.E. Jalowiec, J. Panksepp and P.J. Morgane. Sleep-waking patterns and brain biogenic amine levels in cats after administration of 6-hydroxydopamine into the dorsolateral pontine tegmentum. Pharmac. Biochem. and Behavior 1: 557-567, 1973.

AUTHORS

Peter J. Morgane, Ph.D., and Warren C. Stern, Ph.D.: Laboratory of Neurophysiology and Psychopharmacology, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545



A ROLE FOR DOPAMINE IN THE PSYCHOPHARMACOLOGY OF ELECTRICAL SELF-STIMULATION

Barrett R. Cooper, George R. Breese

INTRODUCTION

The electrical self-stimulation phenomenon, discovered nearly two decades ago by Olds and Milner [21], has received considerable experimental attention in the area of psychopharmacology. In this field, use of self-stimulation was prompted by the possibility that drug-induced changes in responding might provide a means of relating the effects of various centrally acting compounds to biochemical changes in structures and pathways stimulated at the tip of the electrode. Application of this strategy, which employed many classes of centrally acting drugs, resulted in the evolution of what has been referred to as the "catecholamine hypothesis" of self-stimulation [16].

The development of a catecholamine hypothesis of self-stimulation can be traced to several studies demonstrating that monoamine oxidase inhibitors [24], amphetamine [25,29], and other drugs that increase central adrenergic tone resulted in increased rates of self-stimulation of the lateral hypothalamus. Conversely, administration of drugs that could be considered to decrease central adrenergic tone such as reserpine [28], alpha-methyltyrosine [3,25], disulfiram [35], or chlorpromazine [22] was found to decrease lateral hypothalamic self-stimulation. Since intraventricular infusion of norepinephrine reversed the depression of self-stimulation produced by alpha-methyltyrosine and disulfiram [35], attention was focused on the importance of this catecholamine for the maintenance of self-stimulation behavior. Furthermore, several investigators have noted the striking overlap of the anatomical maps of structures and pathways that support self-stimulation behavior with maps of the ascending catecholamine neural systems. From this relationship, it was proposed that noradrenergic neural pathways in brain may mediate, or serve as the anatomical substrate of the reinforcing properties of brain stimulation [30].

More recently, however, attention has shifted to the possibility that dopamine neural systems may also serve some function in self-stimulation of the brain. Evidence favoring a role for dopamine is based on findings that high rates of self-stimulation can be obtained from electrodes placed in the substantia nigra and that an increased rate of dopamine turnover accompanies

reinforcing stimulation of this site [2,16]. It has also been shown that haloperidol and pimozide, drugs thought to block dopamine receptors in brain, decrease self-stimulation of the lateral hypothalamus [19,34], while phentolamine, a noradrenergic blocking drug, does not [19]. Finally, Phillips and Fibiger [23] have related the different biochemical effects of d- and l-amphetamine with regard to norepinephrine or dopamine uptake into synaptosomes [23] to differential effects on self-stimulation of noradrenergic and dopaminergic sites in brain.

In view of the data reviewed above, a current evaluation of a catecholamine hypothesis of self-stimulation would have to include possible roles for both norepinephrine and dopamine neural systems in brain in the mediation of some aspect of self-stimulation behavior. In this paper, we will describe our work with 6-hydroxydopamine and other centrally acting drugs in which we sought to examine further the functional significance of central catecholamine neural systems in the psychopharmacology of self-stimulation of the brain.

EFFECTS OF 6-HYDROXYDOPAMINE ON ELECTRICAL SELF-STIMULATION OF THE LATERAL HYPOTHALAMUS

Following the discovery that 6-hydroxydopamine possessed a neurocytotoxic action on catecholamine containing fibers in brain [5], Stein and co-workers [31] and Breese et al. [9] reported that an acute depression of self-stimulation of the lateral hypothalamus occurred in pargyline treated rats after intraventricular or intracisternal administration. An additional treatment with 6-hydroxydopamine, which resulted in a greater destruction of catecholamine neural processes [5], chronically depressed self-stimulation of the lateral hypothalamus [9], providing further support for the view that catecholamine neural systems were required for the expression of self-stimulation behavior.

The development of procedures to obtain a relatively selective, or "preferential" depletion of either norepinephrine or dopamine content of brain with 6-hydroxydopamine [6] led to the application of these procedures to the study of self-stimulation [7,8,11]. Table 1 summarizes the behavioral effects of these different 6-hydroxydopamine treatments. Acute

TABLE 1

Effects of 6-hydroxydopamine on self-stimulation of the lateral hypothalamus (a)

Electrode Placement (b)	6-OHDA treatment (c)	Days After 6-OHDA Treatment (c) 2	4	8	12
Lateral Hypothalamus	Control	87 ± 10	103 ± 8	100 ± 7	105 ± 10
	NE↓	93 ± 9	98 ± 7	104 ± 8	103 ± 9
	DA ↓	22 ± 11**	39 ± 13**	88 ± 11	92 ± 8
	P+6-OHDA	35 ± 11**	58 ± 15**	74 ± 13*	71 ± 13*

(a) All values are expressed as the mean S.E.M. percent of pretreatment responding for each group. Rats were tested for 15 min a day at a current level which maintained moderate to high rates of self-stimulation. A constant current sine wave stimulus of 0.3 sec duration was delivered for each bar press. There are at least 5 animals in each 6-hydroxydopamine treatment group.

(b) The coordinates for electrode placements are described in Cooper et al. (11).

(c) The 6-hydroxydopamine treatments are described in text and in other publications (11). See Table 3 for catecholamine content obtained after treatment.

** p <.01 Dunnet's t-test. Means within each placement group were compared.

depression of self-stimulation was observed in rats pretreated with pargyline prior to 6-hydroxydopamine to reduce both catecholamines (P+60HDA) and in rats treated with desipramine prior to 6-hydroxydopamine to reduce primarily dopamine in brain (↓DA: Table 1). Reduction of brain norepinephrine by giving repeated small injections of 6-hydroxydopamine (NE↓: Table 1) had little effect on responding. In addition, this experiment was found to replicate the earlier finding that treatment with pargyline plus 6-hydroxydopamine (P+60HDA: Table 1) chronically decreased self-stimulation of the lateral hypothalamus. Rats treated to deplete dopamine eventually recovered pretreatment levels of responding. Characteristic depletions of catecholamines produced by the different 6-hydroxydopamine treatments can be seen in Table 3.

EFFECTS OF ALPHA-METHYLTYROSINE, U-14, 624 AND d-AMPHETAMINE ON SELF-STIMULATION OF THE LATERAL HYPOTHALAMUS AFTER 6-HYDROXYDOPAMINE TREATMENT

Although the previous study indicated that the acute behavioral depression accompanying catecholamine depletion produced by 6-hydroxydopamine appeared to be related to the reduction of dopamine but not norepinephrine, it did not provide the necessary evidence to determine which of the catecholamine systems were implicated in chronic reduction of self-stimulation observed when both catecholamine containing neural systems were destroyed [9]. Previous studies of the behavioral effects of catecholamine depleting drugs in rats treated with 6-hydroxydopamine indicated that damage to catecholamine neural systems resulted in an enhanced sensitivity to the behavioral depressant effects of alpha-methyltyrosine or reserpine [13,14,15]. The above finding suggested that this pharmacological approach could be used to determine the functional significance of these amines for the maintenance of self-stimulation, since a differential sensitivity to additional catecholamine reduction would presumably relate to a functional role in this behavior. Thus, studies were initiated to determine the effects of the tyrosine hydroxylase inhibitor, alpha-methyltyrosine, or the dopamine-beta-hydroxylase inhibitor, U-14,624, on self-stimulation of the lateral hypothalamus in 6-hydroxydopamine treated rats. The results of this experiment (Table 2) indicated that rats with prior depletion of brain dopamine, but not norepinephrine, showed an enhanced reduction in lateral hypothalamic self-stimulation after alpha-methyltyrosine treatment [8,11]. No effect on self-stimulation of the lateral hypothalamus was seen after U-14,624 administration, in spite of a large reduction of norepinephrine content in both control and 6-hydroxydopamine treated rats (Table 3). The finding that U-14,624 did not affect self-stimulation of rats treated to deplete both catecholamines in brain (P+60HDA) was of particular interest, since the data concerning the biochemical effects of U-14,624 in such animals indicated a 95% reduction of whole brain norepinephrine content at the time of testing (Table 3).

TABLE 2

Effects of α-Methyltyrosine (25 mg/kg) or U-14,624 (50 mg/kg) on self-stimulation of 6-hydroxydopamine treated rats with electrodes in the lateral hypothalamus (a)

Placement (b)	(6-OHDA) (b) Group	Percent of Pretreatment Responding (c) α-MT	U-14,624
Lateral Hypothalamus	Control	89 ± 5	102 ± 6
	NE↓	87 ± 9	103 ± 3
	DA↓	50 ± 6**	99.3 ± 4
	P+6-OHDA	22 ± 7**	101 ± 8

(a) Values are expressed as the mean S.E.M. percent of pretreatment responding determined during a 15 minute session on the day to drug treatment. Drug were given in balanced order 1 week apart beginning 16-18 days after 6-hydroxydopamine treatment. At least 6 rats were used In each group.

(b) 6-hydroxydopamine (6-OHDA) treatments are described in Table 1.

(c) α-MT refers to a 25 mg/kg dose of L-α-methyltyrosine given 4 hours before testing, U-14,624 (50 mg/kg) was given 6 hours before testing.

** p< .01 Dunnet's t-test. Means within each placement group were compared.

While the previous experiment suggested the importance of dopamine neural pathways for the maintenance of lateral hypothalamic self-stimulation, failure to obtain evidence that would implicate norepinephrine prompted examination of the effects of d-amphetamine in 6-hydroxydopamine treated rats. Since facilitation of lateral hypothalamic self-stimulation produced by d-amphetamine has been proposed to be related to indirect release of newly synthesized norepinephrine [29,30], it was anticipated that destruction of noradrenergic terminals would attenuate the facilitation of self-stimulation produced by d-amphetamine. Figure 1 shows the results of these experiments.

TABLE 3

Catecholamine content of 6-hydroxydopamine treated rats after α-methyltyrosine (25 mg/kg) or U-14,624 (50 mg/kg)

6-OHDA Treatment	Untreated Rats		α-methyltyrosine		U-14,624	
	NE	DA	NE	DA	NE	DA
Control	100 ± 7	100 ± 5	61 ± 7*	55 ± 7*	27 ± 6*	117 ± 19
NE	39 ± 4	86 ± 7	21 ± 1*	53 ± 6*	8 ± 2*	91 ± 11
DA	87 ± 7	30 ± 5	52 ± 4*	18 ± 4*	27 ± 3*	26 ± 3
P+6-OHDA	26 ± 3	22 ± 2	11 ± 4*	7 ± 3*	5 ± 1*	25 ± 8

(a) Values are expressed as the mean ±S.E.M. percent of catecholamine content of untreated control rats. Catecholamines were determined 4 hours after a-methyltyrosine or 6 hours after U-14.624 to correspond to behavioral testing (See table 1). There are at least 6 rats in each group.

* < P .01 when compared with control rats of the same control or 6-hydroxydopamine treatment condition.

FIGURE 1

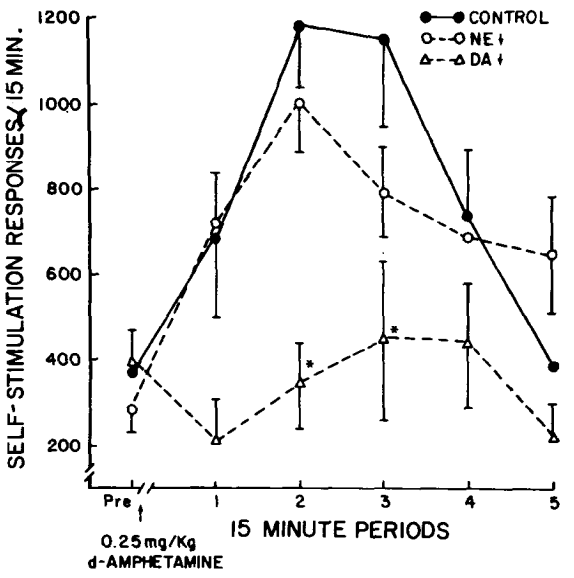


Figure 1: Effects of d-amphetamine on lateral hypothalamic self-stimulation after treatments with 6-hydroxydopamine which reduce norepinephrine (NE+) or dopamine (DA↓) in brain. A current intensity which just maintained responding was determined for each rat prior to treatment with 0.25 mg/kg d-amphetamine sulfate. P<.05 when compared with control.

When the 6-hydroxydopamine treated rats with lateral hypothalamic electrode placements were given d-amphetamine and tested at an intensity that just maintained responding, d-amphetamine sulfate (0.25 mg/kg) failed to facilitate responding of those animals in which brain dopamine was reduced (Fig 1). Rats with brain norepinephrine content depleted by 6-hydroxydopamine, however, displayed significant facilitation of responding which did not differ from control (Fig 1).

In addition to the use of 6-hydroxydopamine, another approach to test the significance of newly synthesized catecholamines for the action of d-amphetamine on self-stimulation was used. Taking a procedure adopted from Hanson's earlier work [17], rats were treated with reserpine (2.5 mg/kg) to eliminate stores of amines and then, 24 hours later, given alpha-methyltyrosine (25 mg/kg) to block synthesis of newly formed norepinephrine. A 1 mg/kg dose of d-amphetamine sulfate was given 1 hr after the synthesis inhibitors. Figure 2 shows that d-amphetamine restored the bar-press response of rats treated with reserpine alone and of rats given

FIGURE 2

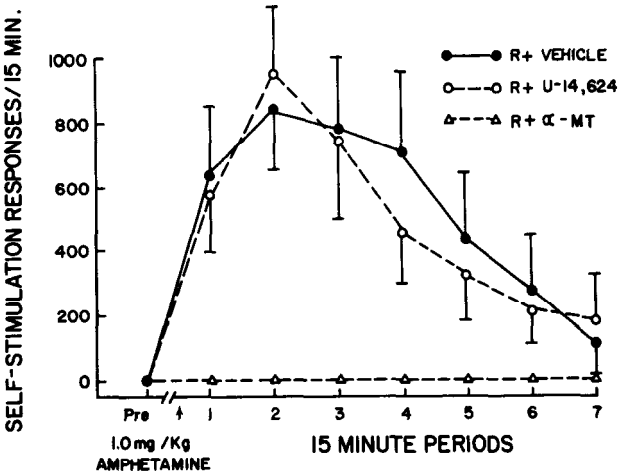


Figure 2: Effects of d-amphetamine on self-stimulation of reserpinized rats with electrodes placed in the lateral hypothalamus. Reserpine (2.5 mg/kg s.c.) was given 24 hours before treatment with either vehicle, U-14, 624 (50 mg/kg) or l-alpha-methyltyrosine (25 mg/kg). D-amphetamine sulfate (1 mg/kg, ip) was given 1 hour after the synthesis inhibitors. Whole brain catecholamine content of rats with similar treatment was determined at the time of amphetamine administration and is given below as a percent of untreated control values: Reserpine = 1.6 + 0.3% norepinephrine; 11.7 ± 2.5% dopamine; Reserpine + U-14, 624 = 0.7 ± 0.01% norepinephrine; 6.1 ± 1.1% dopamine; Reserpine + alpha-MT = 1.1 ± 0.3% norepinephrine; 2.6 ± 1.1% dopamine.

reserpine plus U-14,624. In contrast, alpha-methyltyrosine completely eliminated the ability of d-amphetamine to reinstate lateral hypothalamic self-stimulation in the reserpinized rat. Thus, these results, and those of the previous studies, suggested that dopamine neural systems are implicated in self-stimulaton of the lateral hypothalamus, as well as in the pharmacological alterations of lateral hypothalamic self-stimulation produced by alpha-methyltyrosine, 6-hydroxydopamine, and d-amphetamine.

STUDIES OF THE EFFECT OF BIOGENIC AMINE DEPLETION ON SELF-STIMULATION OF THE LOCUS COERULEUS

Although the work described thusfar was consistent with the hypothesis that dopamine plays a significant role in self-stimulation of the lateral hypothalamus, we were perplexed by the failure to obtain a definite indication that self-stimulation of the lateral hypothalamus involved a noradrenergic component. In order to evaluate further the possible contributions of noradrenergic and dopaminergic pathways to self-stimulation, rats were implanted with electrodes aimed at the locus coeruleus and the effect of a 40 mg/kg dose of alpha-methyltyrosine was compared with the response following a 50 mg/kg dose of U-14,624 [4,12]. When tested 4 hours after alpha-methyltyrosine or 6 hours after U-14,624, only those animals receiving alpha-methyltyrosine displayed reduced responding. In these experiments, U-14,624 reduced norepinephrine by 73%, and alpha-methyltyrosine depleted norepinephrine by 50% and dopamine by 60% suggesting that the reduction of dopamine produced by alpha-methyltyrosine was responsible for the depression of the locus coeruleus self-stimulation [4,12].

DISCUSSION

The results of the experiments described in this paper add to the increasing number of reports concerning a significant role for dopamine in electrical self-stimulation of the brain [16,19,23,34]. Alteration of functional integrity of the dopamine neural systems would appear to be of significance for changes in self-stimulation responding produced *by* 6-hydroxydopamine, alpha-methyltyrosine and d-amphetamine--even when electrodes are placed in the region of the locus coeruleus. Since the locus coeruleus contains no reported dopamine cell bodies, axons, or terminals [33], self-stimulation of the locus coeruleus must involve an indirect activation of dopamine systems. That is to say, the assumption that changes in self-stimulation of a particular site following drug treatment may be related to an action of the drug on the structures stimulated at the tip of the electrode cannot be freely applied in this instance.

It is difficult, at this time, to speculate on how dopamine is involved in self-stimulation. Certainly, the functional significance of the dopamine systems are indicated in many of the behaviors frequently employed in psychopharmacology to evaluate drug effects [7,8,10]. In this context, our current results with self-stimulation, even of the locus coeruleus, are not unique to this measure. Furthermore, the clinical association of Parkinson's disease with reduced content of dopamine in the striatum [18] make it tempting to speculate that the role of dopamine in behavior, as indicated by applications of catecholamine depleting drugs such as 6-hydroxydopamine, alpha-methyltyrosine, or reserpine, may reflect a disturbance in an extra-pyramidal motor control or an integrative system responsible for performance of the self-stimulation response. Such a view, taken to extreme, would suggest that much of the animal work with these agents using operant measures over the past decade may be of primary theoretical relevance clinically to Parkinson's disease and/or related extrapyramidal disorders. There is, however, increasing evidence that the mesolimbic dopamine pathways may be of significance to functions less easily related to extrapyramidal motor control [15,20]. One area for future research in the field of self-stimulation would be to try to associate the deficits produced by dopamine depletion with the nigrostriatal and/or mesolimbic pathways.

If it is difficult to assess the role of dopamine in the maintenance of self-stimulation, it is even more difficult using the data presented in this manuscript to speculate about a role for norepinephrine. Our work, even with locus coeruleus electrode placements did not give a clear indication that this amine participates in the mediation of self-stimulation of the brain. The treatments that preferentially depleted norepinephrine produced no change in responding even for stimulation of the locus coeruleus placement. Finally, no apparent role for norepinephrine neural systems could be defined in the facilitation of self-stimulation produced by d-amphetamine. However, it may be premature to conclude that norepinephrine is not involved in self-stimulation of the brain. With regard to our own experimental procedures, it is quite feasible that 6-hydroxydopamine did not destroy a sufficient number of noradrenergic processes in the appropriate area of brain to produce a clear effect. Problems may also exist in the use of dopamine-beta-hydroxylase inhibitors. Recently, evidence has been reported suggesting that these compounds may result in the release of dopamine from central noradrenergic neurons [1]. It is possible, therefore, that dopamine released from noredrenergic neurons could activate noradrenergic receptors to maintain self-stimulation after U-14,624. Such a possibility, however, would not explain why intraventricular infusions of norepinephrine facilitate self-stimulation while intraventricular infusions of dopamine do not [30,35]. Finally, it is most difficult to understand why there exists such a close correspondence of self-stimulation loci in brain with the central noradrenergic pathways (16,26], if norepinephrine has little, if any, significance for self-stimulation. Nevertheless, the anatomical correlation of self-stimulation with noradrenergic pathways might not necessarily reflect functional relationship for this amine to responding. Clearly, the need for additional research in this area is indicated.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of Gregory Ervin, Joseph Farmer, Susan Hollister, and Edna Edwards. This work was supported by USPHS grants MH-16522, MH-25573 and HD-03110. George R. Breese is a USPHS Career Development Awardee (HD-24585).

REFERENCES

1. Anden, N.E., C.V. Atack and T.H. Svensson. Release of dopamine from central noradrenaline and dopamine nerves induced by a dopamine-beta-hydroxylase inhibitor. J. Neural Transmission 2:92-100, 1973.

2. Arbuthnott, G.W., T.J. Crow, K. Fuxe, L. Olson and U. Ungerstedt. Depletion of catecholamines induced by electrical stimulation of central monoamine pathways. Brain Res. 24:471-483, 1974.

3. Black, W.C. and B.R. Cooper. Reduction of electrically rewarded behavior by interference with monoamine synthesis. Physicol. Behav. 5:1405-1409, 1970.

4. Breese, G.R. and B.R. Cooper. Relationship of dopamine neural systems to the maintenance of self-stimulation. In: Neurotransmitters and Autocoid Balances Regulating behavior. In Press, 1975.

5. Breese, G.R. and T.D. Traylor. Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: evidence for selective degeneration of catecholamine neurons. J. Pharmacol. Exp. Ther. 174:413-420, 1970.

6. Breese, G.R. and T.D. Traylor. Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. Brit. J. Pharmacol. 42:88-99, 1971.

7. Breese, G.R., B.R. Cooper and A.S. Hollister. Relationship of biogenic amines to behavior. J. Psychit. Res. 11:125-133, 1974.

8. Breese, G.R., B.R. Cooper and R.D. Smith. Biochemical and behavioral alterations following 6-hydroxydopamine administration into brain. In: Frontiers in Catecholamine Research, E. Usdin and S. Snyder, editors. Pergamon Press, N.Y., pp. 701-706, 1973.

9. Breese, G.R., J. Howard and P. Leahy. Effect of 6-hydroxydopamine on electrical self-stimulation of brain. Brit. J. Pharmacol. 43:255-257, 1971.

10. Cooper, B.R. and G.R. Breese. Relationship of dopamine neural systems to the behavioral alterations produced by 6-hydroxydopamine administration into brain. In: Neuropsychopharmacology of Monoamines and Their Regulatorv Enzymes, E. Usdin, editor. Raven Press pp 35-368, 1974.

11. Cooper, B.R., J.M. Cott, and G.R. Breese. Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxydopamine treatments. Psychopharmacologia (Berl.) 37:235-248, 1974.

12. Cooper, B.R., G.N. Ervin and G.R. Breese. A role for dopamine in the stimulant effects of amphetamine on locus coeruleus self-stimulation. Pharmacologist 16:249 abs, 1974.

13. Cooper, B.R., L.D. Grant and G.R. Breese. Comparison on the behavioral depressant effects of neuroleptic and biogenic amine depleting agents following various 6-hydroxydopamine treatments. Psychopharmacologia 31:95-109, 1973.

14. Cooper, B.R., G.R. Breese, J.L. Howard and L.D. Grant. Enhanced behavioral depressant effects of reserpine and alpha-methyltyrosine after 6-hydroxydopamine treatment. Psychopharmacologia 27:99-110, 1972.

15. Cooper, B.R., J.L. Howard, L.D. Grant, R.D. Smith and G.R. Breese. Alteration of avoidance and ingestive behavior after destruction of central catecholamine pathways with 6-hydroxydopamine. Pharmacol. Biochem. Behav. 1:639-649, 1974.

16. Crow T.J. Catecholamine-containing neurons and electrical self-stimulation I. A review of some data. Psychol. Med. 2:414-421, 1972.

17. Hanson, L.C.F. Evidence that the central action of amphetamine is mediated via catecholamines. Psychopharmacol. 9:78-80, 1966.

18. Horneykeiwicz, O. Parkinson's disease: From brain homogenate to treatment. Fed. Proc. 32:183-190, 1973.

19. Lippa, A.S., S.M. Antelman, A.E. Fisher and D.R. Canfield. Neurochemial mediation of reward: A significant role for dopamine? Pharmacol. Biochem. Behav. 1:23-28, 1973.

20. Matthysse, S. Schizophrenia: Relationship to dopamine transmission, motor control and feature extraction. In: The Neurosciences. Third Annual Studv Program, F. Schmitt and F.G. Worden, editors. MIT Press, Cambridge, Mass., pp. 733-737, 1964.

21. Olds, J. and P. Milner. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J. Comp. Physiol. Psych. 47:419-427, 1954.

22. Olds, J. and R.P. Travis. Effects of chlorpromazine, meprobamate, pentobarbital and morphine self-stimulation. Pharmacol. Exp. Ther. 128:397,404, 1960.

23. Phillips, A.G. and H. Fibiger, Dopaminergic and noradrenergic substrates of positive reinforcement: Differential effects of d- and l-amphetamine. Science 179:575-577, 1973.

24. Poschel, B. and F. Ninteman. Excitatory (antidepressant) effect of monoamine oxidase inhibitors on the reward system of the brain. Life Sci. 3:903-910, 1964.

25. Poschel, B. and F. Ninteman. Hypothalamic self-stimulation: Its suppression by blockade of norepinephrine biosynthesis and reinstatement by methamphetamine. Life Sci. 5:11-16, 1966.

26. Hitter S. and L. Stein. Self-stimulation of noradrenergic cell group (A6) in locus coeruleus of rats. J. Comp. Physiol. Psych. 85:443-452, 1973.

27. Skinner, J.E.: Neuroscience: A Laboratory Manual. Saunders, London, 1971.

28. Stein, L. Effects and interactions of imipramine, chlorpromazine, reserpine, and amphetamine on self-stimulation: Possible neurophysiological basis of depression. Rec. Adv. Biol. Psychiat. 4:288-309, 1962.

29. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. Fed. Proc. 23:826, 1964.

30. Stein, L. Chemistry of reward and punishment. In: Psychopharmacology. A Review of Progress (1957-1967) D: Efron, editor, PHSP No. 1836. U.S. Government Printing Office, Wash., D.C. PP. 105-123, 1968.

31. Stein, L. and C.D. Wise. Possible etiology of schizophrenia: Progressive damage to the noradrenergic reward system by 6-hydroxydopamine. Science 171:1032-1036, 1971.

32. Taylor, K.M. and S.H. Snyder. Differential effects of d- and l-amphetamine on behavior and on catecholamine disposition in dopamine and norepinephrine containing neurons of rat brain. Brain Res. 28:295-309, 1971.

33. Ungerstedt, U. Stereotaxic mapping of monoamine pathways in the rat brain. Acta Physiologica. Scandanavica. Suppl. 367:1-48, 1971.

34. Wauquier, A. and C.J.E. Neimegeers. Intracranial self-stimulation in rats as a function of various stimulus parameters. II. Influence of haloperidol, pimozide, and pipamperone on medial forebrain bundle stimulation with monopolar electrodes. Psychopharmacologia. (Berl.) 27:191-202, 1972.

35. Wise, C.D. and L. Stein. Facilitation of brain self-stimulation by central administration of norepinephrine. Science 163:299-301, 1969.

AUTHORS

Barrett R. Cooper, Ph.D., and George R. Breese, Ph.D.: Departments of Psychiatry and Pharmacology, Biological Sciences Research Center of The Child Development Institute, University of North Carolina School of Medicine, Chapel Hill, N.C. 27514

AGGRESSION AND THE BRAIN MONOAMINES: WHAT ARE THE ANSWERS, BUT OF MORE IMPORTANCE, WHAT ARE THE QUESTIONS...?[1,2]

Bruce Kenneth Bernard

INTRODUCTION

The study of aggressive behavior and the biochemical mechanisms which may underlie it is not a new endeavor. Comparatively speaking, however, the investigation of the Functional significance of brain monoamines in relation to aggression is of recent vintage. Several discoveries in the early 1950's and 60's, including the introduction of antipsychotic drugs such as the phenothiazines and the development of sensitive analytical techniques for detecting nanogram quantities of postulated neurotransmitters, led to the voluminous brain biogenic amine research experienced during the past 10 years. Yet, the importance of these papers has been diminished by the endless variety of correlations and factors which make generalizations across studies seemingly impossible.

Although there may be many reasons for the apparent lack of cross-study validity? this paper will explore one possibility, non-specificity in questioning. That is, although the various methodologies being employed may provide valid and reliable data, these data may not be generalizable due to basic dissimilarities in the models themselves. Thus, the function of the brain amines may vary across models of aggressive behavior and only after careful evaluation of tne behavioral models themselves can one expect similar relationships to occur. In recent years, workers in our laboratories have investigated a number of models of aggression and their relationship to brain monoamine functioning. These include: isolation-induced aggression [11], natural muricide in the wild rat [10], shock-induced conspecific and ranacide aggression [9,8], hormonal manipulation of natural ranacide [5,4] septal lesion-induced hyperirritability [6] and age related conspecific aggression in mice [7]. It is the expressed purpose of this paper to employ several of these model systems and demonstrate not ohly behavioral similarities and dissimilarities but also similarities and dissimilarities in relation to the brain amines.

Models of aggressive behavior can be compared using a number of criteria. For the purposes of this paper, I will employ two classificatory schematas,

the "eliciting stimulus" and the "neurochemical" models. In the first system, Moyer [28] divides aggressive behavior into six separate models based upon differences in eliciting stimuli: predatory, inter-male, irritable, territorial defensive, maternal and instrumental. In a more recent publication, he reduces this number to five by eliminating territorial defense [27]. In addition to the eliciting stimuli, the behavior is characterized on the limited neurological and endocrinological data available at the time. Thus for example, predatory aggression is thought to be non-androgen dependent and have a neurological basis in the lateral hypothalamus. Intermale aggression, however, appears to involve the septal region and requires the presence of testosterone.

The second schemata, suggested by Reis [30,31] stresses a pharmacological and neurological distinction between two sub-groupings of aggressive behavior, predatory and affective. Predatory aggression may be characterized as: 1) involving the ventral medial tegmentum [15], 2) having no autonomic activation components, and 3) being inhibited by drugs which increase catecholamine (norepinephrine and dopamine) or serotonergic activity, and enhanced by drugs which increase cholinergic functioning [30]. The second subclass of agonistic behavior (affective) can be characterized as: 1) involving the brain central gray, 2) having quite obvious autonomic activation components and 3) being enhanced by drugs which stimulate catecholamine activity and inhibited by drugs which enhance cholinergic or serotonergic activity. Furthermore, as demonstrated in recent articles [4,5] predatory aggression is probably not androgen dependent whereas affective aggression probably is [8].

Using the above classifications, three distinct models of aggressive behavior have been chosen From the numerous ones employed in our laboratories: these are shock-induced fighting, ranacide behavior and septal lesion-induced hyperirritability. As will be reported later in this paper, shock-induced fighting is both androgen-dependent and has autonomic activation components associated with it whereas ranacide is neither androgen dependent nor involves autonomic components. Thus, we have examples of the two classically different models. The third model, hyperirritability, is a mixture of the above two in that it contains autonomic components but is not androgen dependent. Employing these 3 behaviorally distinct models, we will investigate some of the possible relationships which exist between aggression and the brain monoamines.

SHOCK-INDUCED FIGHTING

Fighting between pairs of animals induced by aversive stimulation is one of the more frequently employed models of aggressive behavior cited in the literature. It is a type of "irritable" agonistic behavior as defined by the eliciting stimulus model and "affective" aggression in the neurological classification. Both classificatory schematas suggest androgen dependency for this type of behavior [28,5] and there is supporting evidence for that view [20]. Other reports [16], however, indicate that shock-induced fighting is not manipulable via the hormonal route. Similar discrepancies appear in the literature with regard to hormonal control of brain monoamines. Thus, for example, Stefano and Donoso [35] and others [17] reported an increased hypothalamic NE concentration following castration whereas Sandler [32] and others [21] reported no effect. It occurred to us[3] that variations in the castration-testing interval may be the cause of these apparently contradictory findings. Furthermore, if both shock-induced aggression and the brain amines are androgen dependent, a time-related covariance of these parameters might provide some insight into a possible causal relationship.

Method and Results

Adult male, Sprague-Dawley rats, 170-190 gms. at the start of the experiment, were employed as subjects. Animals were castrated under light ether anesthesia. Control subjects (sham-castrates) underwent identical surgical manipulations as castrated animals; however, the testicles were not removed. Three or six weeks following surgery, animals underwent a series of behavioral tests followed 24 hours later by biochemical analyses of brain amines. The behavioral testing consisted of: a) 3 emotionality testing sessions, 24 hours apart, in an open-field apparatus [18], b) a test

for shock-induced flinch-thresholds [29,3] and c) a test for shock-induced aggressive behavior between pairs of rats [16].

Behavior

The effect of castration on open-field emotionality is shown in Figure 1. A repeated measures analyses of variance [38] showed that castrate subjects traversed significantly more squares than sham subjects but only at the 3 week interval ($p < 0.025$). Analysis of rearing behavior resulted in a pattern identical to that seen in Figure 1 and therefore is not shown. Three other measures of emotionality, latency to enter the first square, boli and grooming behavior were unaltered by castration and there were no significant interactions with either time or trials. This alteration in open-field behavior at the 3 week interval was unrelated to any change in shock-induced aggressive behavior (Figure 2). In the latter behavioral paradigm, castration Failed to alter the fighting pattern at the 3 week interval but did have an effect at the 6 week time period. Analyses of other aggression measures such as number of trials until first fight or First two consecutive fights gave the same results, namely that shams fought significantly more than castrates and took less trials to do so but only at the 6 week interval ($p < 0.05$). These findings could not be attributed to an alteration in Foot-shock sensitivity since (as shown in Figure 3) castration failed to alter flinch-thresholds at either interval.

FIGURE 1

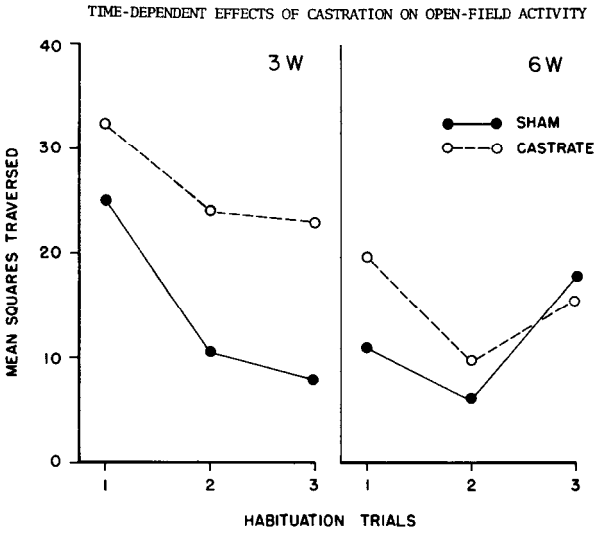


Figure 1: Adult male rats were castrated or sham-castrated. Three or 6 weeks later, the mean number of squares transversed during 3 separate emotionality (habituation) trials spaced 24 hours apart was recorded. Data were analyzed using analyses of variance. Castration had a significant effect only at the 3 week interval ($p<0.025$). Both groups were significantly decreased over time ($p<0.025$). Each point represents the mean value obtained from 12 rats.

Brain Amines

Rat brains were separated into hypothalamus (H) and whole-brain minus hypothalamus (WB-H) and simultaneously analyzed for brain levels of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), norepinephrine (NE) and dopamine (DA) using a modification of previously reported methods [1,14,24]. In addition, the instantaneous rate constants (k), turnover times (TT) and utilization rates (K) for the

FIGURE 2

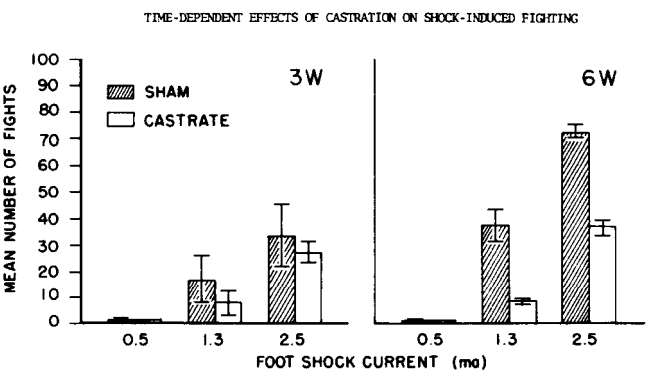


Figure 2: Adult male rats were castrated or sham-castrated. Three or 6 weeks later, animals were tested in pairs for shock-induced aggressive behavior at three shock-intensities (0.5, 1.3 and 2.5 ma). Castration has a significant effect only at the 6 week interval ($p<0.001$). Time significantly increased the aggressiveness of the shams but not the castrate animals ($p<0.001$). Each bar represents the mean value obtained from 12 rats, vertical lines represent ± one standard error.

FIGURE 3

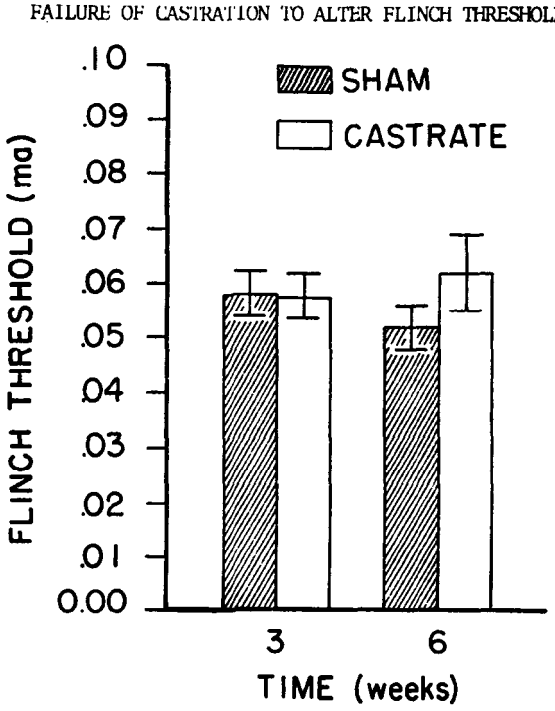


Figure 3: Adult male rats were cas - trated or sham-castrated. Three or 6 weeks Later, animals were tested for shock-induced flinch thresholds. Analysis of variance indicated that neither castration nor time altered this measure. Each bar represents the mean value obtained from 12 rats, vertical bars represent ± one standard error.

TABLE 1

TIME-DEPENDENT EFFECTS OF CASTRATION ON WHOLE BRAIN MINUS HYPOTHALAMUS LEVELS OF 5-HT AND 5-HIAA

CASTRATION-DECAPITATION INTERVAL

	3 WEEK		6 WEEK	
	5-HT	5-HIAA	5-HT	5-HIAA
SHAM	497 ± 7 (10)	478 ± 12 (12)	535 ± 20 (17)	383 ± 13 (17)[a]
CASTRATE	527 ±12 (11)[b]	560 ±12 (12)[c]	697 ±16	413 ± 12 (17)[a]

Concentration expressed in ng/g tissue; Mean ± S.E.
Number of animals in parenthesis.
a $p < 0.0001$ (3 vs 6 weak)
b $p < 0.05$ (sham vs castrate)
c $p < 0.0001$ (sham vs castrate)

TABLE 2

TIME-DEPENDENT EFFECTS OF CASTRATION ON HYPOTHALAMIC NOREPPINERHINE DINAMICS

	Initial concentration (ng/g)	Rate constant, k (h^{-1})	Turnover Time (TT) (h)	Utilization rate (k) (ng/g/h)
3 weeks				
SHAM	1201 ± 38 (3)	0.107 ± 0.022	9.35 [7.75 - 11.76]	129 ± 46
CASTRATE	1558 ± 142 (4)[a]	0.187 ± 0.025[a]	5.36 [5.00 - 6.17]	291 ± 222
6 weeks				
SHAM	1335 ± 37 (3)[b]	0.209 ± 0.041[b]	4.79 [4.00 - 5.95][b]	279 ± 50[b]
CASTRATE	1693 ± 81 (4)[c]	0.239 ± 0.039	4.19 [3.60 - 5.00]	40 ± 138

Number of animals in parenthesis.
a Approaches $p < 0.05$ (sham vs castrate)
b Approaches $p < 0.05$ (3 vs 6 week)
c $p < 0.01$ (sham vs castrate)
[ ] 67 per cent confidence interval

TABLE 3

TIME-DEPENDENT EFFECTS OF CASTRATION ON HYPOTHALAMIC DOPAMINE DINAMICS

	Initial concentration (ng/g)	Rate constant, k (h^{-1})	Turnover Time (TT) (h)	Utilization rate (k) (ng/g/h)
3 weeks				
	334 ± 13 (3)	0.257 ± 0.044	3.90 [3.32 - 4.96]	86 ± 12
CASTRATE	431 ± 30 (5)[a]	0.386 ± 0.034[a]	2.59[b] [2.38 - 2.84]	166 ± 18[c]
6 weeks				
SHAM	608 ± 77 (4)[d]	0.317 ± 0.088	3.15 [2.47 - 4.36]	191 ± 52
CASTRATE	575 ± 43 (5)[d]	0.283 ± 0.054	3.53 [2.97 - 4.36]	163 ± 29

Number of animals in parenthesis.
a Approaches $p < 0.05$ (sham vs castrate)
b Approaches $p < 0.05$ (sham vs castrate)
c $p < 0.01$ (sham vs castrate)
d $p < 0.05$ (3 vs 6 week)
[ ] 67 per cent confidence interval

catecholamines, NE and DA, were obtained using the methods of Brodie et al., [13] and Bernard and Paolino [9], and that data analyzed by analysis of variance [38]. As can be observed in Table 1, brain levels of 5-HIAA were significantly higher in castrate subjects compared to controls only at the 3 week interval while 5-HT levels were significantly greater in castrate animals with the magnitude of the difference increasing in a time-dependent manner. These effects were not seen in the hypothalamus, where castration failed to alter 5-HIAA or 5-HT levels at either time period.

Region specific alterations were also observed for NE dynamics in that castration failed to alter the levels, k, TT or K for this amine in WB-H, yet effected, albeit transitively, NE dynamics in the hypothalamus. As seen in Table 2, both levels and k were increased in the castrate animals at the 3 week interval and although the magnitude of the difference in levels increased at 6 weeks, the rate constants were no longer different. A similar effect on hypothalamic DA rate constants can be seen in Table 3 where castrate subjects had higher levels and rate constants at the 3 week interval but were the same as sham subjects by the 6 week time period. These results are opposite to the findings for WB-H DA analyses, which revealed no alterations in k, TT or K at either interval but did find a significant decrease in DA levels at the 6 week interval (960 ± 29 ng/gm vs 771 ± 43 ng/gm; $p < 0.005$).

Discussion

The results of this study provide evidence that the effect of castration on both brain monoamine dynamics and shock-induced aggressive behavior is time-dependent in nature. Thus, castration altered aggressive behavior at the 6 but not the 3 week interval thereby independently confirming apparently contradictory literature reports [20,16]. Furthermore, these behavioral effects could not be attributed to alterations in general emotionality or altered foot-shock sensitivity since the latter did not change and the former was altered only at the 3 week interval. Similarly, alterations in hypothalamic NE are clearly time-dependent. Thus, experiments which employed castration-analyses intervals of 14 and 21 days [32,21] and reported no change in NE are not necessarily in conflict with those using an interval of 20 and 30 days [35,17] and reporting increased NE. This is further supported by the fact that the results of the 21 day study were just shy of significance at the $p < 0.05$ level.

At first glance, one might conclude that castration led to a time-dependent decrease in aggressive behavior and levels of WB-H DA. Careful analyses however revealed that in both instances, castration per se had no effect on either measure (3 vs 6 week). Rather, in both cases age-dependent increase was observed in the sham subjects. It would appear therefore that the effect of castration was the inhibition of this natural, maturational process, thereby fixating the animal at the level achieved at the time of castration. These data suggest a definitive pattern of covariance between decreased aggressive behavior and: 1) increased brain 5-HT and 2) decreased brain DA. An additional covariance pattern was observed between increased open-field activity and brain levels of 5-HIAA and DA. This sequence of events may be interpreted in several ways, one of which is a simplistic causal relationship between brain amines (where 5-HT has inhibitory and NE and DA excitatory functions) and affective behavior. A more complex, yet justified conclusion relates these behaviors to relative aminergic dominance, where a single brain aminergic neuronal system is of functional importance only when considered in relation to the other brain amine systems. These interpretations will be more fully explored in the GENERAL DISCUSSION section of this paper.

RANACIDE BEHAVIOR

Ranacide, or the killing of frogs, is a second type of aggressive behavior but one which has anatomical and pharmacological properties which are quite distinct from shock-induced fighting. It has been classified as "predatory" aggression in both classificatory schemes in that: 1) it is a very stereotyped behavior pattern elicited by a limited range of stimuli [2], and 2) the hehavior is facilitated by cholinergic stimulation of the lateral hypothalamus (see Ref. 2). Furthermore, it has been reported that rats will seek out, kill and eat frogs under natural conditions Eibesfeldt, I., pers. comm., (Eibl-Predatory aggression is described as non-androgen dependent [28,5] and although there is no evidence relating this question to ranacide one might expect the behavior to be non-manipulable via the hormonal route.

The purposes of the following experiments were two-fold: First, to determine if ranacide aggression was androgen dependent in either the adult

male or female rat and second, to determine what if any, relationship might exist between the effects of testosterone on ranacide behavior and the brain monoamines.[4]

Method and Results

Adult male and female Wistar rats, 70-80 days of age at the beginning of the experiments, were employed as subjects. In the first series of experiments, male rats were screened for ranacide behavior in 2 separate, 24 hour testing sessions spaced 24 hr. apart. Animals which killed frogs within 1 min. or less on both trials were defined as killers (K) while animals which failed to kill during both 24 hrs. tests were defined as non-killers (NK). The killers were then separated into 2 experimental and two control groups; some animals received alternate day subcutaneous injections of testosterone propionate (200 ug in sesame oil) for 30 days (K + T) or sesame control injections (K + S) while others were castrated (K + castrate) or sham-castrated (K + sham). The non-killing male animals were divided into two groups and received either testosterone (NK + T) or vehicle injections (NK + S) as described for the killers. Thirty-one days following the beginning of hormonal manipulations, all subjects were given 3 additional ranacide screening sessions 24 hr. apart.

In the second series of experiments, female rats were screened for ranacide on the basis of a single 30 minute session. Subjects which attacked or killed during this period were defined as agressors (A) while animals which failed to do so were called non-aggressors (NA). Aggressors were divided into two groups; those which received the above regimen of testosterone (A + T) or vehicle control injections (A + S). A similar division of the NA group resulted in subjects which received testosterone (NA + T) and their controls (NA + S). Thirty-one days following the+ first injections, all subjects received an additional 30 min. testing session. Following this screening trial, all animals were continued on their appropriate hormonal treatment. Seventy-two hours later, animals were decapitated and their brains assayed for monoamine content.

Behavior

The failure of testosterone administration to significantly alter the median latency to kill in adult male killer rats can be seen in Table 4. Analyses of these data using a Wilcoxon Matched-Pairs Sign-Rank Test [34] indicated a tendency for the latencies of both groups (K + S and K + T) to decrease when Trial 1 was compared to 5 (0.1>p> 0.05). However, a comparison of the trends between the two groups using a Mann-Whitney U test, revealed no significant differences. Identical results were obtained when comparing the latencies of the killer + sham and killer + castrate groups (Table 5). Once again there was a tendency for the latencies of both groups to decrease over trials, yet there was no difference in trends between groups. Thus, neither castration nor testosterone administration per se altered the latency to kill in adult male killer rats.

TABLE 4

ALTERATION IN THE MEDIAN LATENCY TO KILL AS A FUNCTION OF TESTOSTERONE ADMINISTRATION

GROUPS	TRIAL 1	2	3	4	5
Killers + Sesame Oil	45* (50-35) 10*	42 (65-30)	40 (70-15)	30 (65-10)	32** (55-15)
Killers + Testosterone	45 (60-25)	40 (80-20)	40 (300-15)	32 (85-10)	35** (65-15)

* Median latency to kill (seconds)
** .1 > p > .05 Trial 1 vs 5
( ) Range of values
\+ Number of subjects/group

TABLE 5

ALTERATION IS THE MEDIAN LATENCY TO KILL AS A FUNCTION OF CASTRATION

GROUPS	TRIAL 1	2	3	4	5
Killers + Sham-Castration	40* (55-20) 10†	37 (85-20)	30 (50-15)	30 (65-15)	27 (70-15)
Killers + Castration	40 (60-15) 20†	35 (140-15)	37 (300-15)	27 (90-10)	30 (80-5)

* Median latency to kill (seconds)
( ) Range of values
\+ Number of subjects/group

The results of the administration of testosterone to adult male non-killer (NK) provided additional evidence for the non-androgencity of this behavior. Of the 40 rats which failed to kill in the pre-injection trials (1 and 2), not a single animal killed in post-injection trials. The conclusions from these studies are similar to those obtained using adult, female non-aggressive and aggressive rats (Table 6). Although some subjects from both the NK + S and NK + T did kill (2/5 and 4/16, respectively) or attack (5/5 and l2/16, respectively) following the injection sequence, there was no difference between the groups on these measures or the final median latency to attack (6.33 vs 9.53 min., respectively). Similar behavioral analyses showed that adult, female naturally aggressive rats could not be differentiated following either testosterone (A + T) or sesame (A + S) administration (Table 6).

TABLE 6

FAILURE OF TESTOSTERONE ADMINISTRATION TO ALTER ATTACK OR KILL BEHAVIOR IN AGGRESSIVE & NON-AGGRESSIVE FEMALE RATS

		LATENCY TO ATTACK *	
BEHAVIOR	TREATMENT	TRIAL 1	TRIAL 2
NON-AGGCRESSIVE	SESAME OIL [5]	30.00	6.33 (13-2)
	TESTOSTERONE [16]	30.00	9.53 (30-.3)
AGGRESSIVE	SESAME OIL [9]	10.25 (29-5)	9.25[1] (14-2)
	TESTOSTERONE [8]	9.58 (15-1)	2.42[2] (27-1)

* Median latency to attack (min)
( ) Range of values (min)
[ ] Number of animals/group
1 p < 0.02 Trial 1 vs 2
2 p < 0.035 Trial 1 vs 2

Brain Amines

The effect of the above hormonal manipulations on whole-brain levels of 5-HT and DA in these aggressive and non-aggressive adult female rats is shown in Table 7. Data were analyzed using two way analyses of variance. The results indicated no significant effect for behavior, treatment groups or their interaction for either brain 5-HT, or DA. This was not the case for brain levels of NE (Table 8) which was analyzed in a similar manner. Testosterone injected subjects had significantly higher brain NE levels than sesame treated controls (p< 0.001); analysis by behavioral group (non-aggressive vs aggressive) revealed no significant differences.

TABLE 7

FAILURE OF TESTOSTERONE TO ALTER WHOLE-BRAIN SEROTONIN AND DOPAMINE LEVELS IN AGGRESSIVE AND NON-AGGRESSIVE FEMALE RATS

	BRAIN MONOAMINE LEVELS*	
GROUPS	SEROTONIN	DOPAMINE
NON-AGGRESSIVE +	558.9	1382.8
SESAME OIL	± 34.07 (5)	± 23.59 (5)
TESTOSTERONE	573.7	1424.0
	± 24.0 (4)	± 47.4 (4)
AGGRESSIVE +	529.7	1460.9
SESAME OIL	± 15.7 (3)	± 17.02
TESTOSTERONE	515.0	1320.3
	± 19.4 (5)	± 53.3 (5)

* Data expressed as ng amine/gm tissue; Mean ± standard error
Amine levels were analyzed using ANOVA
( ) Number of animals/group

TABLE 8

BRAIN NOREPINERPHINE LEVELS IN ADULT FEMALE RATS AS A FUNCTION OF BEHAVIOR AND HORMONAL TREATMENT

Behavior	Treatment		
	Sesame Oil	Testosterone	
Non-Aggressive	270.3 ± 5.84* (5)	305.5 ± 6.18[1] (4)	285.9 ± 7.36 (9)
Aggressive	287.1 ± 3.43[3] (5)	293.1 ± 2.23[2] (5)	290.1 ± 2.34 (10)
	278.7 ± 4.25 (10)	298.6 ± 3.67[4] (9)	

* Data expressed as ng amine/gm tissue; Mean @ standard error
( ) Number of animals/group
1 p < .01 Non-agg. test. vs non-agg. sesame
p < .05 Non-agg. test. vs agg. sesame
2 p < .05 Agg. test. vs non-agg. sesame
3 p < .05 Agg. ses. vs. Non-agg. ses.
4 p < .001 test. vs sesame

Discussion

The results of the above series of experiments provides evidence in adult male and female, aggressive and non-aggressive, testosterone treated and castrated rats, that ranacide behavior is a non-androgen dependent model of aggressive behavior.

Testosterone was neither an initiator of ranacide behavior in non-aggressive subjects nor was it a promoter of this behavior in naturally aggressive animals. Conversely, testosterone did not inhibit aggressive behavior in animals so inclined. These results are further supported by the fact that in the shock-induced aggression study previously reported, castrated rats which were less aggressive than their sham controls at the 6 week interval (Figure 2), did not differ from each other with respect to ranacide at that same time period (Figure 4).

FIGURE 4

FAILURE OF CASTRATION TO ALTER RANACIDE BEHAVIOR 3 AND 6 WEEKS FOLLOWING SURGERY

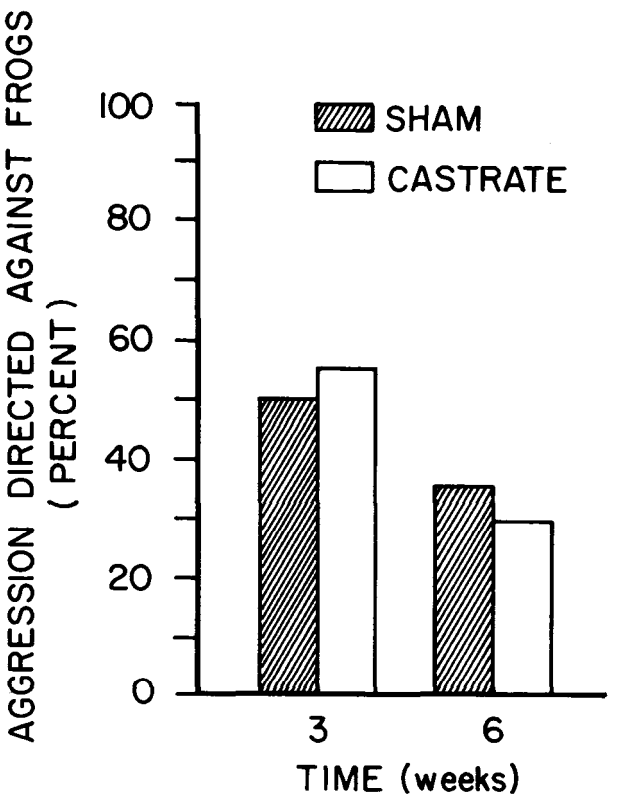


Figure 4: Adult male rats were castrated or sham-castrated and tested for ranacide behavior 3 or 6 weeks later. Data were analyzed using analysis of variance. Neither castration nor time altered either group with respect to this behavior.

SEPTAL LESION INDUCED HYPERIRRITABILITY

The third and final model of aggression to be discussed involves the hyperirritability syndrome which can be induced by stereotaxically placed lesions of the septal region of the brain. The behavior is characterized by increased responsiveness to inanimate object presentation, and an increased likelihood of attack, biting and vocalization during handling [12,6]. This rather stereotyped pattern of the behavior together with its autonomic components is very similar to that described "affective" aggression in the neurochemical classification and "irritable" aggression in the eliciting stimuli model. Hyperirritability differs from shock-induced aggression, however, in that it does not appear to be androgen dependent [26,33]. Thus, hyperirritability has characteristics which are common to both shock-induced and predatory aggression and yet differs in other ways from these same categories.

The purpose of this experiment, as with the previous ones, was to determine if this type of behavior could be correlated with brain monoaminergic changes. Secondly, if the behavior could be related to monoaminergic changes, were these changes similar to that seen in shock-induced aggression or ranacide behavior?

Method and Results

Adult male Wistar rats were employed as subjects in the following experimental paradigm. Animals were screened for emotional reactivity [22] on 3 consecutive days. The final ratings were employed to form 2 behaviorally equivalent groups for surgery. One group received stereotaxically placed, electrolytically induced bilateral lesions of the septum while the other group underwent the same surgical procedure but no current was placed. Forty-eight hours following surgery, animals were again screened for emotional reactivity and then subjected to biochemical procedures designed to evaluate brain monoamine levels and dynamics.

Behavior

The effects of bilateral electrolytic lesions of the septum on emotional reactivity can be observed in Table 9. As previously stated, groups were behaviorally matched prior to surgery (4.97 vs 5.13). The surgical procedure by itself did not effect the postoperative behavior (5.66 vs 5.13, $p > 0.1$), however, the animals which received septal lesions were more than 3 times as reactive as their previous scores (15.15 vs 4.97, $p < 0.01$) and significantly greater than the sham controls ($p < 0.01$).

TABLE 9

THE EFFECT OF BILATERAL ELECTROLITIC SEPTAL LESIONS ON EMOTIONALITY RATINGS[1]

Treatment	Emotionality Ratings[2] Final Preoperative	Postoperative
Septal Lesion	4.97 ± 0.24	15.15 ± 0.013
Sham Control	5.13 ± 0.16	5.66 ± 0.24

1 Data analyzed using a two way, repeated measures ANOVA followed by Norman-Keule analysis for pairs of means. Date expressed as Mean ± Standard Error (N = 15 per treatment).
2 Table values are for ratings taken 24 hr prior to and 48 hr following surgery
3 $p < 0.01$ (Preop. septals vs postop. septals, postop. septals vs postop. shams)

Brain Amines

The effect of septal lesions on brain monoamine levels can be seen in Table 10. Alterations were observed only in the hypothalamus and limbic system where NE levels were significantly reduced in the former ($p < 0.01$) and DA levels reduced in both ($p < 0.05$ and $p < 0.01$, respectively). No alterations were observed in either the cortex or the pons-medulla. Furthermore, there were no changes in the indoleamine system (5-HT and 5-HIAA) in any of the brain parts under consideration. Analysis of NE and DA rate constant (k), turnover time (TT) and utilization rate (K) data revealed no alteration for either catecholamine in any of the brain parts under consideration.

Discussion

The results of this experiment clearly demonstrate the striking behavior effects of septal lesions. The importance of the biochemical profile, that is decreased hypothalamic and limbic DA and hypothalamic NE, is uncertain due to the failure of these lesions to alter catecholamine dynamics (k, TT or K). Furthermore, these biochemical effects are not only anatomically localized, but also confined to the catecholaminergic as opposed to the indoleaminergic neuronal

TABLE 10

THE EFFECTS OF SEPTAL LESIONS ON MONOAMINE LEVELS IN DISCRETE BRAIN REGIONS

	Nonrepinephine*		Dopamine		Serotonin		5-Hydroxyindoleacetic acid	
	Septal	Sham	Septal	Sham	Septal	Sham	Septal	Sham
Limbic (783)	307 ± 16	374 ± 26	2689 ± 50[1]	3317 ± 158	513 ± 24	497 ± 33	521 ± 49	491 ± 74
Hypothalamus (69)	1353 ± 64[1]	1532 ± 56	934 ±301	1214 ± 83	846 ± 81	939 ± 24	921 ± 67	870 ± 28
Cortex (551)	274 ± 19	325 ± 13	698 ± 138	676 ± 121	924 ± 48	896 ± 51	253 ± 16	303 ± 21
Pane-Medulle (205)	456 ± 27	510 ± 38	176 ±19	167 ± 8	692 ± 23	754 ± 20	518 ± 40	483 ± 52
Whole-Brain (1608)	358 ± 202	426 ± 24	1574 ± 76[2]	1924 ± 123	691 ± 35	685 ± 37	446 ± 37	442 ± 51

* Data expressed as ng/g tissue; Mean ± Standard Error ($4 \leq N \leq 5$); Weight of brain tissue (mg)in parentheses.
1 $p < 0.01$ (septal vs sham)
2 $p < 0.05$ (septal vs sham)

systems. The combination of biochemical effects did however alter brain catecholamine to indoleamine ratios, the importance of which will be discussed under GENERAL DISCUSSION.

GENERAL DISCUSSION

The above series of experiments were chosen to demonstrate some of the basic differences between models of aggressive behavior. As stated in the introduction, these behaviors were selected because of their differences in androgen dependency and autonomic components. The importance of this Paper is not, however, these behavioral differences, but rather the divergent effects the treatments had on the brain monoamines in relation to the behaviors.

It has been suggested that the importance of the brain monoamines lay not in the individual neuronal systems, but in the balance between the excitatory and inhibitory transmitters [37,9]. This has been expressed in terms of brain NE/5-HT or DA/5-HT ratios where it is suggested that 5-HT is an inhibitory and NE and DA are excitatory transmitters. When the biochemical alterations in the above experiments are analyzed in this manner, a very interesting pattern emerges. Table 11 summarizes the hypothalamus and whole brain minus hypothalamus monoamine concentrations at 3 and 6 weeks following castration. In addition, the percent change in NE/5-HT and DA/5-HT ratios are shown. The hypothalamic values at the 3 week interval are initially negative (indicating a relative catecholaminergic dominance) but are greatly reduced at the 6 week interval. In contrast, the whole-brain minus hypothalamus is dominated by the serotonergic system at both intervals. Thus, castration which is associated with a less aggressive animal (shock-induced) as compared to controls, is also associated with an increase in serotonergic dominance. These data would agree with the postulate inhibitory role for 5-HT and excitatory role for NE and DA.

TABLE 11

ALTERATIONS IN THE NE/5-HT AND DA/5-HT RATIOS IN SAT HYPOTHALAMUS AND WHOLE BRAIN MINUS HYPOTHALAMUS 3 AND 6 WEEKS FOLLOWING CASTRATION

Tissue	Time (Weeks Following Castration)	Amine Concentration Changes After Castration*	Hypothesized Transmitter % Changes in Ratios produced by castration** NE/5-HT	DA/5-HT
Hypothalamus	3	NE (Increase) DA (Increase) 5-NT (No change)	-77.2	-21.1
	6	NE (Increase) DA (No Change) 5-HT (No Change)	-21.1	- 0.3
Whole Brain (minus hypothalamus)	3	NE (No Change) DA (No Change) 5-HT (Increase)	+21.0	+13.2
	6	NE (No Change) DA (Decrease) 5-HT (Increase)	+13.9	+68.9

* Comparing Sham Castrate at the same time interval.
* Calculated by substracting the Sham from the Castrate ratio at the same time interval.

If the biochemical data obtained in the ranacide experiments are analyzed in terms of monoamine ratios, the resulting pattern can be seen in Table 12. As was observed in the shock-induced aggression study, the aggressive animals had significantly higher NE/5-HT ratios than the non-aggressive ones. Likewise, the subjects receiving exogenous testosterone had significantly higher ratios than those receiving sesame oil control injections. These two factors, aggression and testosterone, appear to be of equal importance inasmuch as the ratios for the aggressive sesame treated animals were the same as the non-aggressive testosterone treated subjects and both values were located between the other two groups. These results may be interpreted as indicating no functional relationship between the changes in aminergic ratios and ranacide behavior since the former was altered and the latter remained unchanged.

TABLE 12

ALTERATIONS IN WHOLE-BRAIN NOREPINEPHRINE/SEROTONIN RATIOS IN AGGRESSIVE & NON-AGGRESSIVE FEMALE RATS AS A FUNCTION OF TESTOSTERONE ADMINISTRATION

Behavior*	Treatment		
	Sesame	Testosterone	
Non-Aggressive	.463 ± .010 (4)	.535 ± .030 (4)	.499 ± .020 (8)
Aggressive	.545 ± .014 (3)	.572 ± .023 (5)	.562 ± .016 (8)
	.498 ± .017[1] (7)	.556 ± .019 (9)	

* Data expressed as Mean ± standard error
( ) Number of animals/group
1 $p < .05$ Sesame vs Testosterone
2 $p < .02$ Aggressive vs Non-aggressive

While this interpretation may in fact be valid, there exist at least two other possible explanations. First, although the change in NE/5-HT ratios was qualitatively the same in both the shock-induced aggression and ranacide studies, the alterations were not quantitatively the same. The changes observed in the former study were of greater magnitude than those observed in the latter experiment. Thus, in spite of the fact that the changes in the second study were statistically significant, they may not have been large enough to be of functional significance. This of course implies the existence of a minimal change in the NE/5-HT ratios which is required prior to any possible effect on the behavior. The second alternative interpretation involves the means by which the change in ratios occurs rather than the size of the change. The NE/5-HT ratios of the shock-induced castrated animals were lower than the sham-castrated controls due to differences in 5-HT concentrations, not NE. This was not the case in the ranacide study, where changes in NE/5-HT ratios resulted from NE rather than 5-HT alterations. Thus, although the concept of a necessary balance between the brain aminergic systems may be valid, the value of this concept may depend upon changes induced by 5-HT alterations, whereas small changes in NE may be relatively unimportant.

The third and final experiment described herein related the behavioral alterations (hyperirritability) induced by septal-lesions to the monoaminergic effects at the same 2 day post-surgery time period. The results of analyzing the data (in terms of ratios) as just described, can be seen in Table 13. It should be noted that the only alteration observed was in the limbic system NE/5-HT ratio where the septal animals were significantly lower, more serotonergic, than the sham controls. This result is opposite to that which might have been expected since a more reactive animal might conceivably have been more catecholaminergic (excitatory) in nature as compared to to the controls. Furthermore, this change in NE/5-HT was induced by NE rather than 5-HT alterations.

TABLE 13

DIFFERENCES IN BRAIN NE/5-HT RATIOS FOLLOWING SEPTAL LESIONS*

Brain Region	NE/5-HT		DA/5-HT	
	Septal Lesion	Sham Control	Septal Lesion	Sham Control
Limbic System	0.591 ± 0.038[1]	0.755 ± 0.039	5.29 ± 0.27	6.69 ± 0.57
Hypothalamus	1.64 ± 0.11	1.63 ± 0.06	1.15 ± 0.13	1.30 ± 0.11

* Data are expressed as Mean ± Standard Error (N = 5)
1 $p < 0.05$ (sham vs septal)

In light of the above, what can we conclude about the basic question, "What is the functional significance of the brain monoamines with regard to aggressive behavior?" Surely, if these experiments have demonstrated anything, it is that the question has become naive. Its generality is obsolete, rendered so by the progressive discrimination of subcategories of aggressive behavior. It is one of those starting points from which scientific inquiry works away, towards a more specific and definitive plateau of understanding. Its major weakness is not in what it asks, but in what it fails to ask due to the generality of its underlying assumption, that aggression is an undifferentiated mode of behavior with which brain monoamine function might be expected to correlate. To be of value, the question must be more particular: "How do the brain amines relate …to ranacide, muricide and irritable aggression, …to predatory, irritable and spontaneous aggression, …or even to differences within a single (irritable) aggression?" These questions must be asked and indeed are now being asked [25, 19 and 36, respectively]. These studies and the ones reported herein suggest that a more fruitful approach to the study of the brain amine aggressive behavior hypothesis may lie in the careful elaboration of the characteristics of the behavioral model being employed and determinations of how alterations in these basic characteristics relate to the different relationships with the brain amines.

FOOTNOTES

1. This paper was presented at the tnirteenth annual meeting of the American College of Neuropsychopharmacology, in San Juan, Puerto Rico, 1974.

2. Parts of this work were supported by the following agencies: University of Connecticut Research Foundation, National Institutes of Mental Health, and Public Health Service.

3. The shock-induced aggression study described herein was co-authored with Dr. Ronald M. Paolino, present address: Department of Psychiatry and Behavioral Sciences, University of Oklahoma Sciences Center, Oklahoma City, Oklahoma, 73190.

4. The ranacide study in female rats was performed with the technical assistance of Miss Beverly Cocrane and Mr . David Furlano.

5. The septal lesion study was co-authored with Dr. David A. Yutzey and Mr. James R. Berchek, present address: Department of Psychology, University of Connecticut, Storrs, Connecticut 06268.

REFERENCES

1. Ansell, G.B. and M.F. Beeson. A rapid and sensitive procedure for the combined assay of noradrenaline, dopamine and serotonin in a single brain sample. Analyt. Biochem. 23: 196-206, 1968.

2. Bandler, R. Cholinergic synapse in the lateral hypothalamus for the control of predatory aggression in the rat. Brain Res. 20: 409-424, 1970.

3. Beatty, W. and P. Beatty. Hormonal determinants of sex differences in avoidance behavior and reactivity to electric shock in the rat. J. Comp. Physical. Psychol. 73: 446-455, 1970.

4. Bernard, B.K. Testosterone manipulations: effects on ranacide aggression and brain monoamines in the adult female rat. Submitted for publication.

5. Bernard, B.K. Frog killing (ranacide) in the male rat: lack of effect of hormonal manipulations. Physiol. Behav. 12(3): 405-408, 1974.

6. Bernard, B.K., J.R. Berchek and D.A. Yutsey. Alterations in brain monoaminergic functioning associated with septal lesion induced hyperreactivity. Pharmac. Biochem. Behav. 3: 121-126, 1975.

7. Bernard, B.K., E.R. Finkelstein and G. M. Everett. Alterations in mouse aggressive behavior and brain monoamine dynamics as function of age. Physiol. Behav., In press.

8. Bernard, B.K. and R.M. Paolino. Temporal effects of castration on emotionality and shock-induced aggression. adult male rats. Physiol. Behav., 14: 201-206, 1975.

9. Bernard, B.K. and R.M. Paolino. Time-dependent changes in brain biogenic amine dynamics following castration in male rats. J. Neurochem. 22: 951-956, 1974.

10. Bernard, B.K. and R.M. Paolino. The use of biochemical correlates for the prediction of muricidal behavior in the wild rat. Fed. Proc., 32(3): 785, 1973.

11. Bernard, B.K. and R.M. Paolino. Brain norepinephrine levels and turnover rates in castrated mice isolated for 13 months. Experientia a (2): 221-222, 1973.

12. Brady, J.V. and W.J.H. Nauta. Subcortical mechanisms in emotional behavior: affective changes following septal forebrain lesions in the albino rat. J. Comp. Physiol. Psychol. 46: 339-346, 1953.

13. Brodie, B., E. Costa, A. Dlabac, N.H. Neff and W.H. Smookler. Application of steady state kinetics to the estimation of synthesis rate and turnover time of tissue catecholamines. J. Pharmac. Exp. Ther. 154: 493-498, 1966.

14. Chang, C.C. A sensitive method for spectrophotofluorometric assay of catecholamines. Int. J. Neuropharmac. 3: 643-649, 1964.

15. Chi, C. and J. Flynn. Neural pathways associated with hypothalamicallv elicited attack behavior in cats. Science 171: 703-705, 1971.

16. Conner, R.L. and S. Levine. Hormonal influences on aggressive behavior. In: Aggressive Behavior, edited by S. Garrattini and E. Sigg. New York: Wiley, 1969, pp. 150-163.

17. Coppola, J.A. The apparent involvement of the sympathetic nervous system in the gonadotrophin secretion of female rats. J. Reprod. Fert. 4: 35-45, 1968.

18. Denenberg, V.H. Open-field behavior in the rat: what does it mean. Ann. N.Y. Acad. Sci. 159: 852-859, 1969.

19. Eichelman, B.S. and N.B. Thoa. The aggressive monoamines. Biol. Psychiat. 6(2): 143-164, 1973.

20. Hutchison, R., R. Ulrich and N. Azrin. Effects of age and related factors on the pain aggression reaction. J. Comp. Physiol. Psychol. 59: 365-369, 1965.

21. Hyyppa M. and M. Valavaara. The effect of castration and hypophysectomy on the content of noradrenaline and serotonin in the hypothalamus of the rat. Experientia 15: 193-194, 1970.

22. King, F.A. Effects of septal and amygdaloid lesions on emotional behavior and condition avoidance responses in the rat. J. Nerv. Ment. Dis. 126: 57-63, 1958.

23. Knutson, J.F. and M.T. Hyman. Predatory aggression and irritable aggression: shock-induced fighting in mouse-killing rats. Physiol. Behav. 11: 113-115, 1973.

24. Maickel, R., R. Cox, J. Saillant and F. Miller. A method for the determination of serotonin and norepinephrine in discrete areas of rat brain. Int. J. Neuropharmac. 7: 275-281, 1968.

25. McLain, W.C., B.T. Cole, R. Schrieber and D.A. Powell. Central catechol- and indolanine systems and aggression. Pharnacol. Biochem. Behav. 2: 123-126, 1974.

26. Montgomery, R.L., M.K. Berkut, E.F. Grubb and D.L. Westbrook. Hormonal influence on behavior in brain lesioned male rats. Physiol. Behav. 7: 107-111, 1971.

27. Moyer, K.E. Territorial aggression: is this concept useful? Totus Homo, 4(3): 104-117, 1972.

28. Moyer, K.E. Kinds of aggression and their physiological basis. Behav. Biol. 2(A): 65-87, 1968.

29. Pare, W.P. Age, sex and strain differences in the aversive threshold to grid shock in the rat. J. Comp. Physiol. Psycho1 69: 214-218, 1969.

30. Reis, D.J. Central neurotransmitters in aggression. Aggression. Res. Publ. Ass. Res. Nerv. Ment. Div., 52: 119-148, 1974.

31. Reis, D.J. The relationship between brain norepinephrine and aggressive behavior. Res. Publ. Ass. Res. Nerv. Ment. Dis. 50: 266-297, 1972.

32. Sandler, R. Concentration of norepinephrine in the hypothalamus of the rat in relation to the estrous cycle. Endocrinology, 83, 1383-1386, 1968.

33. Seggie, J. Effect of adrenalectomy or gonadectomy on affective behavior changes following septal lesions in the rat. J. Comp. Physiol. Psychol. 74: 11-19, 1971.

34. Siegal, S. In: Nonparametric Statistics For the Behavioral Sciences, New York: McGraw-Hill, 1956.

35. Stefano, F. and A. Donoso. Norepinephrine levels in the rat hypothalamus during the estrous cycle. Endocrinology 81: 1405-1406, 1967.

36. Stolk, J.M., R.L. Conner, S. Levine and J.D. Barchas. Brain norepinephrine metabolism and shock-induced fighting behavior in rats: differential effects of shock and fighting on the neurochemical response to a common foot shock stimulus. J. Pharmacol. Exp. Ther. 190: 193-209, 1974.

37. Tagliamonte, A., P. Tagliamonte, G. Gessa and B. Brodie. Compulsive sexual activity induced by p-chlorophenylalanine in normal and pinealectonized rats. Science 166: 1433-1435, 1969.

38. Winer, B.J. In: Statistical Principles in Experimental Design. New York: McGraw-Hill, 1971.

AUTHOR

Bruce Kenneth Bernard, Ph.D.: Division of Pharmacology and Toxicology, University of Connecticut, Storrs, Connecticut 06268

PHARMACOGENETIC STUDIES OF THE SEROTONERGIC SYSTEM IN ASSOCIATION WITH CONVULSIVE SEIZURES IN MICE

Benson E. Ginsburg, Paul Y. Sze

INTRODUCTION

The effects of genetic differences on behavioral capacities can be demonstrated in a variety of ways. If we confine our interest to mammals, including man, such differences have been demonstrated in inbred strains of mice and rats for behaviors as diverse as sexual performance [11,14,21], learning [1], aggression [7,13,15,25], alcohol preference [6,20], alcohol and drug tolerance [2,17,28], maternal behavior [23], susceptibility to seizures [10,13,19], various forms of ataxia [3], reactions to stress [29], differential effects of identical lesions [19], and many others [26]. These researches depend upon finding strain differences in behavioral parameters and demonstrating, by means of appropriate genetic analyses based on crosses among strains that differ in these parameters, that the differences are genetically based. Selection experiments in genetically variable populations can accomplish the same thing--namely, the demonstration that there is a genetic underpinning for the behavior. In human pedigrees, the major techniques used to establish a genetic etiology include genealogical analyses, twin studies, sibship analyses, and karyotyping.

Once the demonstration of a genetic basis is achieved, its further characterization in genetic terms, far from being an exercise in genetic esoterica, becomes relevant for the understanding of the mechanisms involved, and thereby of the possibility and direction of pharmacological intervention. The same behavioral or morphological phenotype often occurs in a given species on a variety of genotypic bases [9,13]. Each genetic route to the common phenotype may represent a different link in a chain of causal mechanisms, as would be the case with a series of enzymatic reactions where the product of one becomes the substrate for the second. Another possibility is that of distinct alternative mechanisms. A single phenotype may, therefore, include a diversity of underlying mechanisms, which if parsed genetically, could be distinguished for purposes of differential treatment.

One of our seizure-prone mouse mutants, for example, responds well to pre-treatment with monosodium glutamate, while another does not [8]. Both share a common phenotype. Aggressive behavior in male mice is influenced by a number of genetically distinguishable predispositions. Regimes that will change this behavior in one strain will not necessarily produce a similar change in another [12,15]. Even where a given gene can be shown to affect a process and both the genetic substitution and the associated process are highly correlated with a behavior, the associated mechanism may not be causal for the behavior, but may represent one of several pleiotropic actions of the *gene* that may be producing its effect on the behavior via another action [19]. Since we will be dealing with such data in this presentation, the burden of proof that a mechanism, in this case serotonergic, that is associated with both genetic and behavioral differences, is involved in the mediation of the genetic differences and does, in fact, represent an essential mechanism in the development of the behavior, devolves upon us.

In the light of massive data demonstrating genetic control of enzymatic processes, it becomes reasonable to hypothesize that genetic effects on behavioral capacities might be mediated, in part, by genetic variation in enzyme systems involved in the synthesis and degradation of the major neural transmitters. In investigating this possibility, it must be recognized that key variations in transmitter characteristics can be localized both temporally (to a particular period in development) and spatially (to a particular CNS structure). Such genetically based developmental differences have been described in our laboratory for glutamic acid decarboxylase in relation to the production of GABA, and for tryptophan hydroxylase, the rate-limiting enzyme in the synthesis of serotonin [4,16,27]. These enzyme differences are correlated with genetic differences in predisposition to audiogenic seizures and with aggressive behavior in male mice. No similar relationships involving tyrosine hydorxylase were found. In addition, seizures induced by the administration of alcohol during early development and seizures induced by alcohol withdrawal in older mice, are both associated with serotonergic effects [17,28]. The effect of chronic alcohol treatment on tryptophan hydroxylase activity could not be demonstrated with adrenal-ectomized mice, but was restored by the injection of corticosterone. Glucocorticoids are similarly required for the effects of ethanol withdrawal seizures to occur [28].

MATERIALS AND METHODS

Animals

The following mouse stocks were used in these studies: DBA/1/Bg, C57BL/6/Bg, C57BL/10/Bg and CD1 (Swiss albino).

Audiogenic seizures

Seizure tests were conducted in an insulated chamber 38 cm. x 43 cm. x 38 cm. high in which an Edwards-Lungen house bell (7.6 cm. in diameter) was used to provide a sound level of 95-105 dB (in re, 2 x 10^{-4} dyne/sq. cm.) at the floor of the chamber. The mouse was placed in the chamber and its activity, defecation and urination were recorded for one minute. The bell was then activated for 90 seconds. Convulsive seizures occurring during the test period were recorded. Seizures were scored for severity, latency scores were recorded, and seizure incidences were calculated as the percent of mice exhibiting convulsive seizures out of the total number of mice tested. Incidences in Table 1 represent the percent of mice that convulsed when tested on four consecutive days beginning at 28 days of age. Other incidences are based on a single test.

Ethanol administration

Ethanol was administered in the drinking water (10% v/v) of mice *on* ad lib normal diets in those experiments where effects on the offspring while *in* utero and/or during lactation were under investigation [11]. For studies of withdrawal seizures of adult mice, male CD1 and C57BL/10/Bg mice, each bred in our own colony, were housed in groups of five. The CD1 mice were 70 ± 7 days of age, and the C57BL/10/Bg mice *were* 66 ± 8 days of age on the first day of ethanol treatment. All mice were fed a liquid diet as the only

source of food and water. The liquid diet for the ethanol groups consisted of 60% Shape-Metracal (Mead-Johnson & Co.), 6% (v/v) ethanol, 0.9% NaCl, and water. The average daily consumption of liquid diet containing ethanol was 7-9 ml. per mouse, an ethanol intake of approximately 340-440 mg./day. Control mice received the identical liquid diet containing an isocaloric amount of sucrose instead of ethanol. CD1 mice received ethanol for 21 days, and C57BL/10/Bg mice received it for 14 days, since these periods result in maximal seizure induction after withdrawal for each strain. At the end of the experimental period, ethanol was withdrawn at 9 a.m. and the control diet was substituted. Seven hours later, the mice were tested for audiogenic seizures (28).

Aggression test

Litters were weaned at 29 days of life and males were individually isolated until days 50-52 post partum, when they were tested for intermale, intragenotype aggressive behavior [24,25]. Isolation-induced aggression was measured in dyadic encounters on three consecutive days. Male pairs were usually littermates and partners remained constant over the three trials. Aggression testing occurred in a "neutral" cage (16 x 26.5 x 11.5 cm.), divided in half by an opaque partition and covered at the top by a 6.4 mm. wire mesh. No attempt was made to eliminate odors from the test apparatus or room between pairs. Testing took place between 8 a.m. and 6 p.m. in a room used only for that purpose. Overhead fluorescent lighting delivered 94 ftc at the level of the test cage floor. One member of each pair was tail-marked with a Magic Marker® so that the observer could readily distinguish them. Animals were handled with padded forceps throughout the testing period. At 52 days of life, each mouse was weighed to the nearest 0.5 grams. Pairs differing in weight by more than 3.0 g were dropped from the sample.

Subject animals were placed one on each side of the partition and the partition removed after an initial five minute adaptation period. Behavioral records were kept per 20 second interval for a test duration of ten minutes. Agonistic acts scored consisted of tail rattling, flank biting, wrestling, chasing and full attack. Latency values from the time the partition was lifted to the first agonistic act and to the first full-blown attack were also recorded. Composite "any aggression" scores were determined for each pair by Dr. M. Selmanoff [24] by counting "1" for each 20 second interval during which any agonistic act upon an opponent occurred. If both members of a pair exhibited one or all of these behaviors in an interval, the score was "2" for that interval. Hence, with thirty 20-second intervals Per day, the maximum pair score was 180 for three days. Scores for each pair were summed on three trials, combined with those of the other pairs for each genotype and averaged.

Enzyme assays

Tryptophan hydroxylase was assayed using the method of Ichyama et al. [18]. Tyrosine hydroxylase was measured using the method of Nagatsu and co-workers [22]. Enzyme activities were determined in whole brain homogenates.

RESULTS

Tryptophan hydroxylase activity was measured from the fourth day of life post partum until the thirty-third day in the C57BL/10/Bg and the DBA/l/Bg strains by Dr. J.A. Diez, working in our laboratory [43]. The results are illustrated in Figure 1, where the solid lines represent the enzyme activity of the C-57 strain and the broken lines that of the DBA/1 strain [4,5]. Measurements were made in whole brain homogenates of males only. The C57BL/10/Bg mice exhibited a higher level of enzyme activity throughout the test period than that shown by the DBA/1/Bg males. They also exhibited a faster rate of increase in enzyme activity during development than did the DBA/1's. Since the brain concentrations of tryptophan precursor were not different in the two strains, our data, indicate that these C57BL mice have a greater capacity to synthesize serotonin than do their DBA/1 counterparts. This activity difference is most likely due to the amount of enzyme present rather than to a structural difference in the enzyme molecule which would affect its activity, since the K_m of the enzyme for tryptophan is identical in the two

FIGURE 1

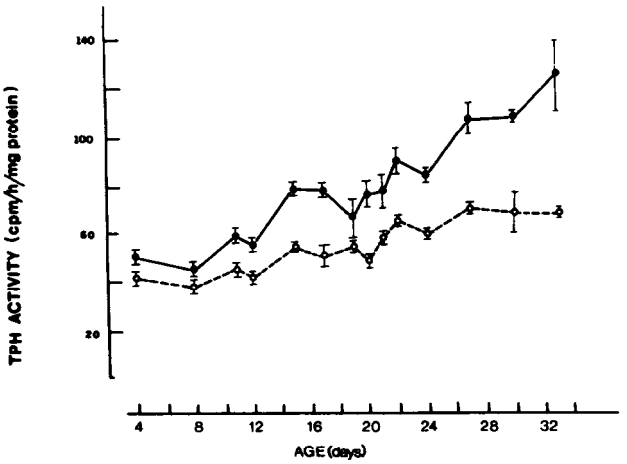


Figure 1: Developmental changes of brain tryptophan hydroxylase activity in C57BL/10/Bg (●) and DBA/1/Bg mice (o). (From: Dies, J. A., P. Y. Sze and B. E. Ginsburg, 1975. Difference in brain tryptophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5:94-95). (See, also, reference 25).

FIGURE 2

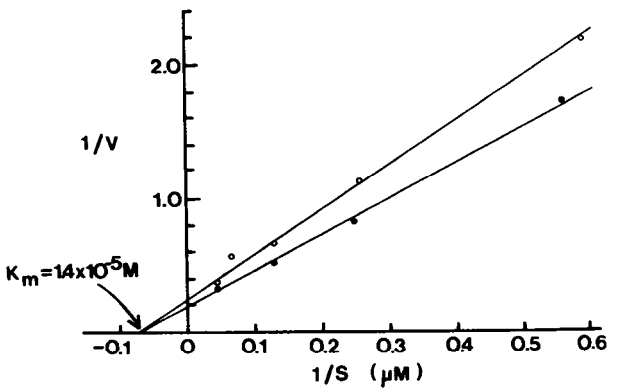


Figure 2: Apparent K_m of tryptophan hydroxylase for tryptophan in brain homogenates for C57BL/10/Bg (●) and DBA/1/Bg (o) mice. (From: Diez, J. A., P. Y. Sze and B. E. Ginsburg, 1975. Difference in brain tryptophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5:94-95). (See, also, reference 251.

strains at 30 days of age (see Figure 2). The progressive increase in the straine difference is also consistent with this interpretation [4,5 op. cit.].

As Diez (op. cit.) pointed out, the results in Figure 1 could also be attributed to an excess of tryptophan hydroxylase activator in brains of the C57DL/10/Bg mice, or, conversely, to an enzyme inhibitor contained in the brains of the DBA/1/Bg animals. If the first alternative were true, a mixture of C57 and DBA brain homogenates would be expected to provide a higher level of enzyme activity than the algebraic average of the two strains. If the DBA's had an enzyme inhibitor, a mixed-strain homogenate would have lower activity than the algebraic average of the two strains. When such homogenates were actually mixed, the expected algebraic average was obtained, suggesting that the strain difference is not due to either an excess of activators or inhibitors [19 and Table 2]. Table 1 summarizes the results obtained for tryptophan hydroxylase activity at 30 days of age for male mice of the strains already mentioned, as well as those of the C57BL/10/Bg strain and for the various F_1 crosses among them. These results are arranged in ascending order relative to their enzyme activities. Differences between genotypes separated by a dashed line are statistically significant. The right hand column represents the incidence of audiogenic seizures in these strains and crosses, where the tests were carried on four consecutive days beginning at day 28 post partum. The highest enzyme activity was exhibited by the two C57BL strains, which did not differ significantly from each other. When these were crossed, however, the resulting F_1 had a significantly lower incidence than either parental strain. F_1's between DBA/1/Bg and either C57BL strain were significantly lower than those obtained from the high parental strains or the F_1 between them and were significantly higher than those obtained from DBA/1/Bg. All were somewhat lower than the expected mid-parental value, suggesting possible partial dominance of the DBA/1 characteristic. Reciprocal F_1's between the C57BL/10/Bg and the DBA/1/Bg strains were not appreciably different from each other. There is, therefore, no suggestion of sex linkage or of a maternal effect [4,5].

TABLE 1

Ginsburg and Sze

Correlation Between Brain Tryptophan Hydroxylase (TPH) Activity and Audiogenic Seizures[1]

Genotype*	(pmole/hr/mg prot.)[b]	Audiogenic Seizures[c] (% Incidences)
DBA/1	4.17 ± 0.05 (16)	81%
$B6D1F_1$	4.54 ± 0.08 (6)	48%
$BIODIF_1$	4.66 ± 0.06 (8)	57%
$DIBIOF_1$	4.67 ± 0.07 (8)	57%
$BlOB6F_1$	5.00 ± 0.09 (16)	0%
C57BL/10	5.40 ± 0.07 (16)	0%
C57BL16	5.44 ± 0.08 (16)	0%

[a] All mice were males, one month of age.

[b] Mean ± S.E.M. with number of nice in parentheses. Values separated by dotted lines are significantly different ($p < 0.05$) from adjacent values.

[c] Each value from at least 300 mice tested.

[1] From: Diez, J.A., P.Y. Sze and B.E. Ginsburg, 1975. Difference in brain tryptophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5:94-95.

FIGURE 3

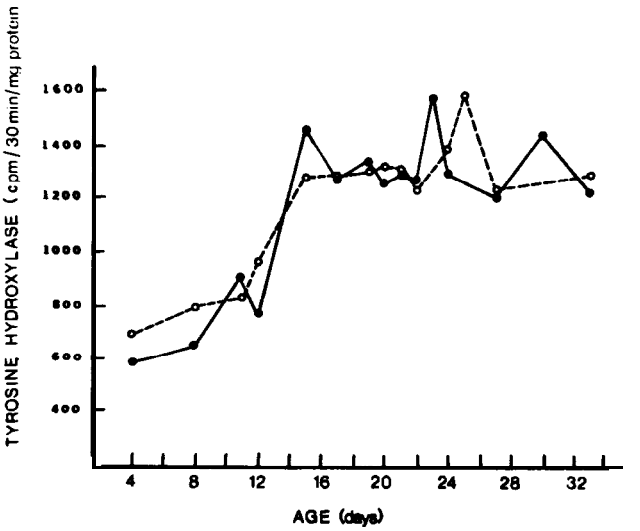


Figure 3: Developmental changes of brain tyrosine hydroxylase activity in C57BL/10/Bg (●) and DBA/1/Bg (o) mice. (From: Dies, J. A., P. Y. Sze and B. E. Ginsburg, 1975. Difference in brain tryptophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5:94-95). (See, also, reference 25).

TABLE 2

Effect of Mixing DBA/1/Bg and C57BL/10/Bg Brain Homgenates

Homogenate Source	TRYPTOPHAN HYDROXYLASE Mean	TRYPTOPHAN HYDROXYLASE Triplicate Values	5-HTP DECABBOXYLASE Mean	5-HTP DECABBOXYLASE Triplicate Values
DBA/1/Bg	1043	(1012, 1094, 1024)	22.6	(22.8, 22.7, 22.5)
C57BL/10/Bg	1344	(1341, 1359, 1331)	21.6	(21.7, 21.6, 21.5)
DBA + C57	1174	(1202, 1156, 1165)	22.4	(22.5, 22.2, 22.6)
Calculated Mean of DBA and C57	1193		22.1	

Tryptophan hydroxylase activity is DPM/100 mg tissue. 5-HTP decarboxylase activity is (DPM/100 mg tissue) x 10^{-3}.

Eight brains from each strain were pooled and homogenized in 4.0 volumes of 0.3 M sucrose. Triplicate assays were performed on each homogenate and a 1:1 mixture of the two (DBA + C57).

Enzyme activity was determined as described under Methods, but the 5-HTP decarboxylase substrate did not contain exogennnous factor.

[1] From: Diez, J. A., P. Y. Sze and B. E. Ginsburg, 1975. Difference in brain tryprophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 2:94-95. (see, also, reference 25).

Figure 3 summarizes the data we have obtained on a developmental basis measuring tyrosine hydroxylase activities for the DBA/1/Bg and C576L/10/Bg strains over the same time period studied for tryptophan hydroxylase [19]. Only male mice were used for these assays, which were performed with whole brain homogenates. No significant differences in tyrosine hydroxylase activity were found. By comparison with tryptophan hydroxylase, the developmental pattern is different. In both strains, the increase in tryptophan hydroxylase was essentially linear and began at approximately the eighth day of life. By contrast, tyrosine hydroxylase activity remained low until about day 12 and increased sharply to day 16 after which it remained approximately level. The two enzymes, therefore, appear to be regulated quite differently, both with respect to the levels of enzyme activity between the two strains and the pattern of developmental change occurring within the strains. At 30 days of age, no significant differences in tyrosine hydroxylase activity were seen between the two strains in question and the C57BL/6/Bg strain or the F_t's between the DBA/1/Bg and either C57BL/10/Bg or C57BL/6/Bg strains (Fig. 3).

TABLE 3

Ginsburg and Sze

Correlation Between Brain Tryptophan Hydroxylase (TPH) Activity and Aggression[1]

Genotype[a]	TPH Activity[b] (pmole/hr/mg prot.)	Aggression Score[2]
DBA/1	4.17 ± 0.05 (16)	35.4
$B6D1F_1$	4.54 ± 0.08 (6)	5.2
$B10D1F_1$	4.66 ± 0.06 (8)	19.8
$D1B10F_1$	4.67 ± 0.07 (8)	10.7
$B10B6F_1$	5.00 ± 0.09 (16)	7.1

[a] All mice were males, one month of age

[b] Mean ± S.E.M. with number of mice in parenthesis. Values separated by dotted lines are significantly different ($p<0.05$) from adjacent values.

[1] From : Diez, J.A., P.Y. Sze and B.E. Ginsburg, 1975. Difference in brain ryptophan hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5:94-95.

[2] From: Selmanoff, M.K., J.E. Jumonville, S.C. Maxson and B.E. Ginsburg, 1975. Evidencce for a Y chromosomal contribution to an aggressive phenotype in inbred mice. Nature 253:529-530.

So far as the behavioral measures are concerned, neither seizures (Table 1) nor aggression (Table 3) differences between the strains tested and their F_t hybrids are related to tyrosine hydroxylase activities since these were the same in all cases tested. With respect to both audiogenic seizures and aggression scores, there appears to be an inverse correlation with tryptophan hydroxylase activity. These correlations suggest that the serotonergic system may be involved in both of these behaviors. Since the behaviors are not independent of each other, it is not surprising that similar correlations with the serotonergic systems are obtained for both.

In addition to investigating the relationship between tryptophan hydroxylase activity, tyrosine hydroxylase activity, and genetic susceptibility to audiogenic seizures, the relationship of tryptophan hydroxylase activity to audiogenic seizures resulting from withdrawal from ethanol was investigated using CD1 mice and C57BL/10/Bg mice [28]. The results are summarized in Figures 4-7. Under the conditions of our test, neither of these strains exhibits convulsive seizure activity when exposed to sound. After withdrawal from ethanol, however, both strains are markedly susceptible to sound induced seizures. The susceptibility to withdrawal seizures is, however, dependent upon corticosterone, since adrenalectomized mice of either strain exhibit only a small fraction of the withdrawal seizures that would be expected were their adrenals intact. If, however, the adrenalectomized mice are given daily subcutaneous injections of corticosterone (0.5 mg/mouse) during the period of chronic ethanol administration, the seizure incidence is restored to that of the intact animal. Tryptophan hydroxylase activities occurring in brain homogenate and brain P_2 fractions obtained from mice undergoing ethanol treatment with and without adrenalectomy shows a similar dependence on the intact adrenal or on substituted corticosterone. Thus, the association between this aspect of the Serotonergic system and the associated behavior is either causal, in the sense that the glucocorticoids affect the seizure incidence via the serotonergic system, or dependent upon a common mechanism involving glucocorticoids [28].

FIGURES 4 and 5

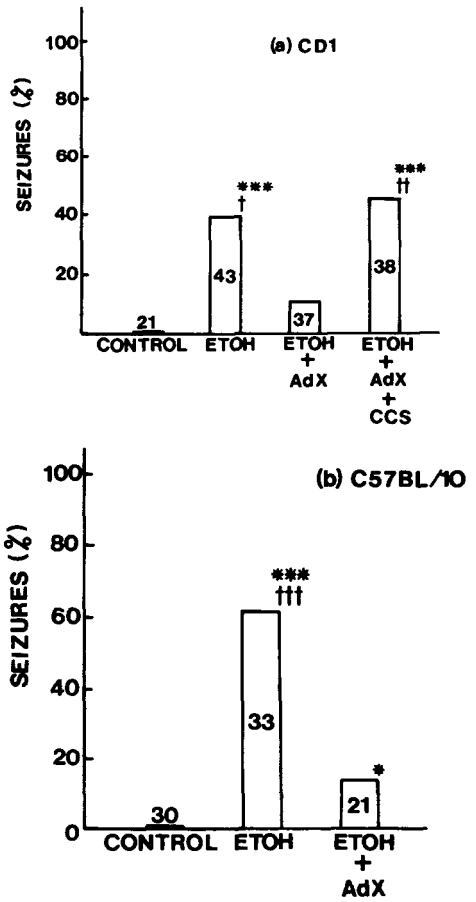


*Figure 4 and Figure 5: Incidence of audiogenic seizures during withdrawal in CD1 (Fig. 4) mice and C57BL/10 (Fig. 5) mice receiving various treatments. Control: intact mice without ethanol administration; ETOH: intact mice with chronic ethanol administration; ETOH + AdX: adrenalectomized mice with chronic ethanol administration; ETOH + AdX + CCS: adrenalectomised mice with chronic ethanol administration and daily subcutaneous injections of corticosterone (0.5 mg/mouse). Seizure incidence is expressed as percent of mice exhibiting convulsive seizures in the total number of mice tested (indicated by the number in each bar). Significance of differences: *p<0.05, ***p<0.001, as compared with the control group; † p<0.05, †† p<0.01, ††† P<0.001, as compared with the ETOH + AdX group. (From: P. Y. Sze, J. Yanai and B. E. Ginsburg, 1974. Adrenal glucocorticoids as a required factor in the development of ethanol withdrawal seizures in mice. Brain Research 80: 155-159.*

FIGURES 6 and 7

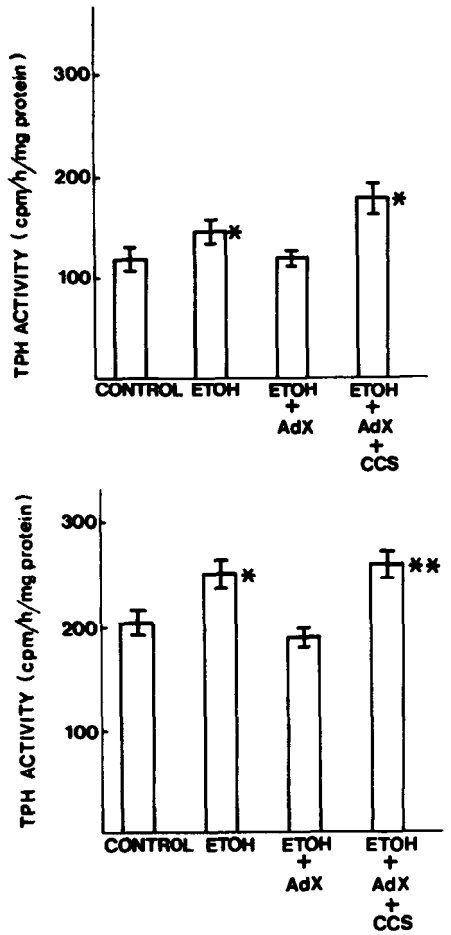


*Figure 6 and Figure 7: Tryptophan hydroxylase (TPH) activities in (Fig. 6) brain homogenate and (Fig. 7) brain P_2 fraction from mice under various treatments. Control: intact mice without ethanol administration: ETOH: intact mice with chronic ethanol administration for 2 weeks; ETOH + AdX: adrenalectomized mice with ethanol administration for 2 weeks: ETOH +AdX + CCS: adrenalectomized mice with chronic ethanol administration and daily subcutaneous injection of corticosterone (0.5 mg in 0.1 ml sesame oil/mouse). The enzyme activity is expressed as counts/min/h/mg protein. Each value is the mean from 6-8 animals ± S.E.M. * p<0.01 as compared with the control, ** p<0.001 as compared with the control. (From: P. Y. Sze and L. Neckers, 1974. Requirement for adrenal glucocorticoid in the ethanol-induced increase of tryptophan hydroxylase activity in mouse brain. Brain Research 72: 375-378).*

The association between sound induced seizures and the serotonergic system is further strengthened by the data summarized in Figure 8. Here, seizures were induced in C57BL/10/Bg mice by administering ethanol to them via the mothers during intrauterine development and the first two weeks of lactation. Using a total of 124 mice, 22% were found to be seizure susceptible when tested at 30 days of age by comparison with control samples showing no seizure activity. Figure 8 summarizes the effects of aminergic drugs in modifying activity.

FIGURE 8

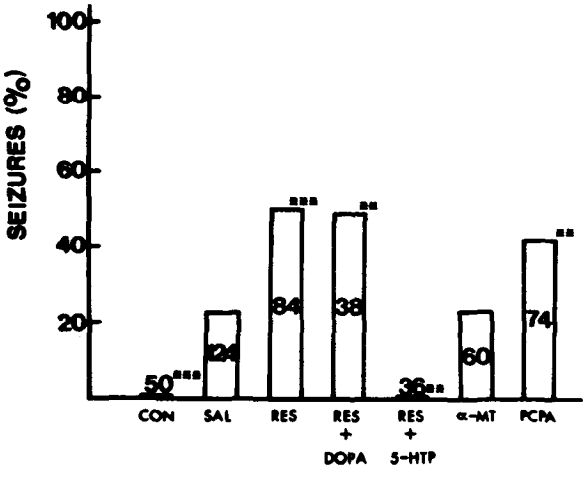


*Figure 6: Effects of drugs on ethanol-induced audiogenic seizures in mice. All mice were offspring from C57BL/10/Bg parents receiving 10% ethanol in drinking water throughout the entire period of pregnancy and 14 days post-partum. Only controls (CON) were not exposed to ethanot. Audiogenic seizures were tested starting at 29 days of age. Reserpine (RES): 2 mg/kg at 2.5 hr before testing; DOPA: 200 mg/kg at 0.5 hr; 5-hydroxytryptophan (5-RTP): 200 mg/kg at 0.5 hr; alpha-methyl-p-tyrosine (alpha-MT): 200 mg/kg at 6 hr; alpha-chlorophenylalanine (PCPA): 300 mg/kg at 24 hr. Saline (SAL) was given to ethanol controls. Numbers in each bar indicate the number of mice in each group. ** p<0.01, *** p<0001 for the deviation from the saline injected ethanol control. (From: J. Yanai, P. Y. Sze and B. E. Ginsburg, 1375. Effects of aminergic drugs and glutamic acid on audiogenic seizures induced by early exposure to ethanol. Epilepsia 16: 67-71).*

the ethanol induced susceptibility to these seizures. When reserpine was injected 2-1/2 hours prior to testing, the seizure incidence increased from 22%, observed in alcohol treated saline controls, to 48%. The injection of DOPA one-half hour prior to testing did not change the incidence of the seizures in the reserpine treated animals. If, however, 5-HTP was injected one-half hour before testing in the reserpine treated group, the seizures were completely prevented. Alpha-MT injected at six hours prior to testing did not modify the seizure incidence induced by early ethanol administration. A single injection of PCPA administered 24 hours after the first test increased the seizure incidence to 42%, a level that was not statistically different from that obtained with reserpine. These results suggest that the serotonergic system is very likely associated with the production or the expression of seizures induced by alcohol administered to mouse pups during early development. They also failed to demonstrate any association between these events and the catecholaminergic system [17].

DISCUSSION

The biogenic amines (serotonin, dopamine and norepinephrine) have been implicated in the regulation of a number of behavioral measures for which the DBA and C57BL strains of mice differ. Since the biogenic amines may be functionally regulated at several levels (including synthesis, storage, release, post-synaptic action, re-uptake and catabolism), genetic variation in any of these processes could prove to be a mechanism by which differential gene action might regulate behavioral function. We chose to study the regulation of synthesis using as endpoints the activities of the rate-limiting biosynthetic enzymes --tryptophan hydroxylase (for serotonin) and tyrosine hydroxylase (for the catecholamines). Since an enzyme is a gene product, genetic variations in enzyme activity could reflect differences in the gene's coding for the enzyme, or in regulatory genes. They might also be due to other metabolic differences, such as hormones, which could affect gene expression or enzyme activity. The association between genes, behavior, and biogenic amines were here studied developmentally. Such an approach

should provide information useful in identifying critical or sensitive periods for biochemical and behavioral differentiation that involve genetic regulation. In general, and in pharmacological studies, it is assumed that changes in neurotransmitter turnover rates have a functional significance. Measures of in vivo synthesis rates are often taken as indicators of turnover. Since C57BL mouse brains have the capacity to synthesize more serotonin than do DBA/1's, we would predict that the amine has a higher turnover rate in the C57. In attempting to interpret the functional significance of this difference, one must consider the possibility that the C57BL mice may require a higher turnover to maintain the same functional levels that DBA/1 mice achieve with a lower rate of turnover. It should also be noted that the lack of genetic differences in whole brain tyrosine hydroxylase activity reported here does not preclude the possibility of significant differences in particular brain regions.

With respect to the ethanol induced increase of tryptophan hydroxylase activity, this was shown to be accompanied by a marked increase in the turnover rate of serotonin. If changes of synaptic transmitter systems are the molecular basis for the rebound hyperexcitability resulting in audiogenic seizure susceptibility after prolonged neuronal depression by alcohol, the case for the involvement of the serotonergic system as a component in the development of withdrawal seizures is strengthened.

With respect to seizures induced by alcohol administered transplacentally and via the mother's milk, it would appear from our results that these are associated with the serotonergic system and not with possible catecholamine differences. Reserpine, which enhances such seizures, decreasees both brain serotonin and catecholamines, presumably by depleting the presynaptic storage depots. The fact that administration of 5-HTP, the precursor of serotonin, not only reversed the effect of reserpine, but completely prevented the occurrence of seizures, further supports the idea that a serotonergic mechanism is involved in the ethanol induced audiogenic seizures. This argument is strengthened by the fact that the administration Of PCPA (a drug that depletes brain serotonin by inhibiting tryptophan hydroxylase activity) also enhanced the seizure incidence to a level comparable to that obtained with reserpine. By contrast, drugs acting on the catecholaminergic system were ineffective in modifying either the effect of reserpine or the seizure incidence. The administration of DOPA, viewed here in its role as precursor of catecholamines, did not reverse the effect of reserpine, as did 5-HTP. The blocking of catecholamine biosynthesis by alpha-MT, an inhibitor of tyrosine hydroxylase activity, similarly produced no effects on the seizure incidence.

While the mechanism by which the serotonergic system acts in the production of susceptibility to audiogenic seizures is not identified in these experiments, its essential role in the seizure mechanism is indicated by its association with genetic variation in seizure incidence, with ethanol withdrawal seizures, with the effects of adrenalectomy and corticosterone substitution on ethanol withdrawal seizures, and by its association with seizures induced by the administration of ethanol during early development.

With respect to aggressive behavior, we have demonstrated a significant negative correlation between tryptophan hydroxylase activity and the aggression scores of male mice of various genotypes. The genetic basis for differences in aggressive behavior is complex and other factors, such as male hormone have been implicated in the expression of aggressive behavior, as well as associated with the genetic differences in these mouse strains [5,6,7,8,22,26]. It has also been shown that there are possible causal interactions between aggressive behavior and seizures and that they may even have overlapping genetic predetermination. It is possible, therefore, that the serotonergic mechanism is a component of both behaviors and that those genetic factors that are common to both exert their effect via the serotonergic system.

ACKNOWLEDGMENT

The authors gratefully acknowledge the support of NIAAA grant AA 00297-03, PHS grant RR-00602, a grant from The Grant Foundation, Inc., and a grant from The Connecticut Research Foundation.

REFERENCES

1. Bovet, D., F. Bovet-Nitti and A. Oliviero. Genetic aspects of learning and memory in mice. Science 163: 139-149, 1969.

2. Catz. C. and S.J. Yaffe. Strain and age variation in hexobarbital response. Pharmacol. Exp. Ther. 155: 152-156, 1967.

3. Coggeshall, R.E., C.J. D'Amato, M.A. Brodbine and S.P. Hicks. Developmental neuropathology of genetic mutant mouse (paralytic). Fed. Proc. 20: 330, 1961.

4. Diez, J.A. Genetic variation and the role of adrenal function in the regulation of serotonin and catecholamine synthesis in developing and adult mouse brain. Ph.D. dissertation, Department of Biobehavioral Sciences, The University of Connecticut, 1973.

5. Diez, J.A., P.Y. Sze and B.E. Ginsburg. Difference in brain tryptophen hydroxylase activity in C57BL and DBA mouse genotypes. Behav. Genet. 5: 94-95, 1975.

6. Fuller, J.L. Measurement of alcohol preference in genetic experiments. Comp. Physiol. Psychol. 51: 85-88 1964.

7. Ginsburg, B.E. and W.C. Allee. Some effects of conditioning on social dominance and subordination in inbred strains of mice. Physiol. Zool. 15: 485-506, 1942.

8. Ginsburg, B., S. Ross, M.J. Zamis and A. Perkins. Some effects of 1(+)-glutamic acid on sound-induced seizures in mice. J. Comp. Physiol. Psychol. 44: 134-141, 1951.

9. Ginsburg, B.E. Genetics as a tool in the study of behavior. Persp. Biol. Med. 1: 397-424, 1958.

10. Ginsburg, B.E. and D.S. Miller. Genetic factors in audiogenic seizures. In: Psychophysiologie, Neuropharmacologie et Biochimie de la Crise Audiogene. Paris: Centre National de la Recherche Scientifique, No. 112, 1963, pp. 217-225.

11. Ginsburg, B.E. Coaction of genetical and nongenetical factors influencing sexual behavior. In: Sex and Behaviour, edited by F. Beach. New York: John Wiley and Sons, 1965, pp. 53-75.

12. Ginsbrug, B.E. All mice are not created equal -- recent findings on genes and behavior. Soc. Serv. Rev. 40: 121-134, 1966.

13. Ginsburg, B.E. Genetic parameters in behavior research. In: Behavior-Genetic Analysis, edited by J. Hirsch. New York: McGraw-Hill, 1967, pp. 135-153.

14. Ginsbrug, B.E. Breeding structure and social behavior of mammals: a servo-mechanism for the avoidance of panmixia. In: Genetics. Biology and Behavior Series, edited by D. Glass. New York: Rockefeller University Press and Russell Sage Foundation, 1968, pp. 117-128.

15. Ginsburg, B.E. Genotypic factors in the ontogeny of behavior. Science and Psychoanalysis 12: 12-17, 1968.

16. Ginsburg, B.E., J.S. Cowen, S.C. Maxson and P.Y. Sze. Neurochemical effects of gene mutations associated with audiogenic seizures. In: Progress in Neuro-Genetics, edited by A. Barbeau and J.R. Brunette. Amsterdam: Excerpta Medica, 1969, pp. 695-701.

17. Ginsburg, B.E., J. Yanai and P.Y.

Sze. A developmental genetic study of the effects of alcohol consumed by parent mice on the behavior and development of their offspring. In: Proceedings of the Fourth Annual Alcoholism Conference of the National Institute on Alcouhol Abuse and Alcoholism, in press.

18. Ichyama, A., S. Nakamura, Y. Nichizuka and 0. Hayaishi. Tryptophan-5-hydroxylase in mammalian brain. Adv. Pharmacol. 6A: 5-17, 1968.

19. Maxson, S.C. The effect of genotype on brain mechanisms involved in audiogenic seizure susceptibility. Ph.D. dissertation, Department of Psychology, University of Chicago, 1966.

20. McClearn, G.E. and D.A. Rodgers. Differences in alcohol preferences among inbred strains of mice. Quart. J. Stud. Ale. 20: 691-695, 1959.

21. McGill, T.E. Sexual behavior in three inbred strains of mice. Behaviour 14: 341-350, 1962.

22. Nagatsu, T., B.G. Levitt and S. Udenfriend. Tyrosine hydroxylase: the initial step in norepinephrine biosynthesis. J. Biol. Chem. 238: 2910-2917, 1964.

23. Paschke, R.E., V.H. Denenberg and M.X. Zarrow. Mice reared with rats: An interstrain comparison of mother and "aunt" effects. Behaviour 38: 315-331, 1971.

24. Selmanoff, M.K. Genetic variation and the role of testosterone in the development and adult expression of aggression in inbred mice. Ph.D. dissertation, Department of Biobehavioral Sciences, The University of Connecticut, 1974.

25. Selmanoff, M.K., J.E. Jumonville, S.C. Maxson and B.E. Ginsburg. Evidence for a Y chromosomal contribution to an aggressive phenotype in inbred mice. Nature 253: 529-530, 1975.

26. Sprott, R.L. and J. Staats. Behavioral studies using genetically defined mice--a bibliography. Behav. Genet. 5: 27-82, 1975.

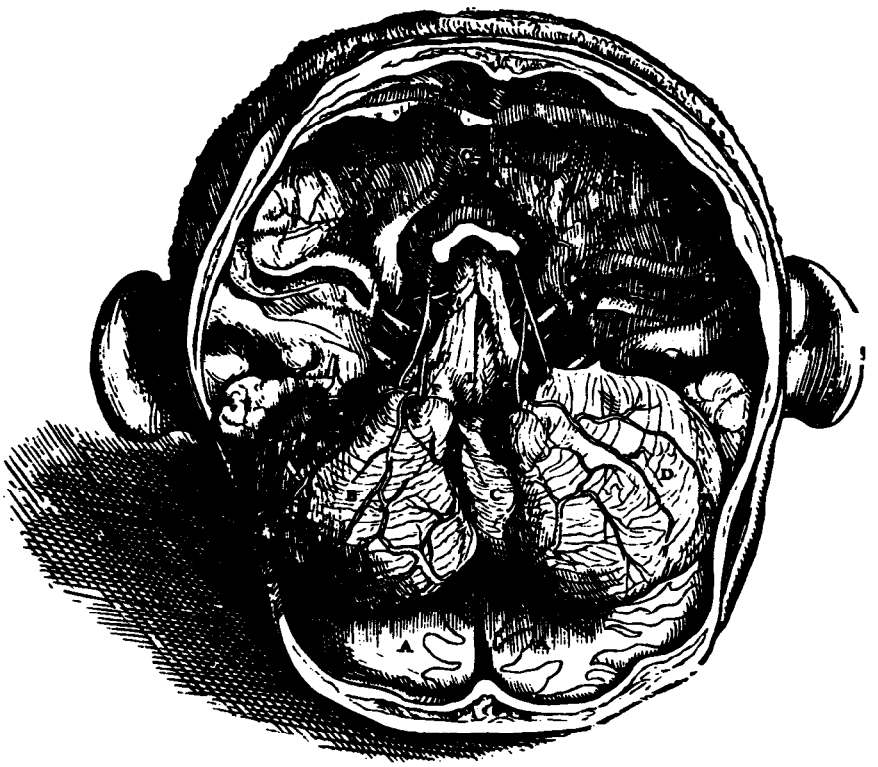
27. Sze. P.Y. Neurochemical factors in auditory stimulation and development of susceptibility to audiogenic seizures. In: Physiological Effects of Noise, edited by B.L. Welch and A.S. Welch. New York: Plenum Press, 1970, pp. 259-269.

28. Sze, P.Y. and L. Neckers. Requirement for adrenal glucocorticoid in the ethanol-induced increase of tryptophan hydroxylase activity in mouse brain. Brain Research 72: 375-378, 1974.

29. Treimar, D.M., D.W. Fulker and S. Levine. Interaction of genotype and environment as determinants of antiesteriol response to stress. Develop. Psychobiol. 3: 13l-l40, 1970.

ATUHORS

Benson E. Ginsburg, Ph.D., and Paul Y. Sze, Ph.D.: Behavioral Genetics Laboratory, Department of biobehavioral Sciences, The University of Connecticut, Storrs, Connecticut 06268



A MODEL FOR THE NEUROBIOLOGICAL MECHANISMS OF ACTION INVOLVED IN LITHIUM PROPHYLAXIS OF BIPOLAR AFFECTIVE DISORDER

Arnold J. Mandell, Suzanne Knapp

INTRODUCTION

At a recent conference sponsored by the Neurosciences Research Program of MIT, Mogens Schou challenged the basic researchers who are investigating the neurobiological actions of lithium by listing some requirements he felt must be met in order for their findings to be relevant to the drug's clinical actions. He pointed out the necessity of using doses that produce blood levels of drug reasonably comparable to clinically effective levels. He noted the necessity of differentiating acute and chronic effects because efficacious treatment requires adaequate time as well as adequate drug. His most interesting caveat, from our point of view, relates to the clinical work [1,2] showing that following sufficient treatment (sometimes many months) lithium is equally prophylactic against the recurrence of manic and depressive states: Any proposed mechanism of action involving a single transmitter system (e.g. stabilization of membrane reactivity [3,4]; inhibition of catecholamine release [5]; or alterations in serotonin biosynthesis [6-8]) would necessarily be capable of affecting transmitter function in both directions, i.e. increasing and decreasing neurotransmission. In this paper we describe our work *on* the acute and chronic effects of lithium administration on the serotonin biosynthetic apparatus in the brains of rats and include a model by which our results may be understood. In brief, we introduce the possibility that lithium treatment results in reduced adaptive capacities in the serotonergic biosynthetic system in brain that nonetheless allow the system to continue to function within a restricted but normal range. We also discuss the relationship between our animal model and the recently reported data about serotonergic metabolites in the CSF of manic-depressive patients treated with lithium which appear to mirror our findings with regard to time course and directions.

In our first report on the effects of short- and long-term lithium administration on the serotonergic biosynthetic systems in rat brain [9], we said that lithium initially stimulated high affinity synaptosomal uptake of radioactive tryptophan in striatum, which apparently increased the rate at which radioactive tryptophan was converted to serotonin by the striate synaptosomes. These changes were followed by a decrease in soluble tryptophan hydroxylase activity in the

midbrain, and a delayed return of the synaptosomal conversion rate to control levels while the high affinity uptake of tryptophan continued at its accelerated rate. We speculated that a feedback-regulated decrease in intrasynaptosomal enzyme originated in the serotonergic cell bodies of the midbrain and was transported by axoplasmic flow to the nerve endings of the striate cortex. The artificial pteridine cofactor that we used in those experiments (2-amino-4-hydroxy-6,7-dimethyl-5,6,7,8-tetrahydropteridine, $DMPH_4$) was relatively inefficient, and we could not directly measure solubilized tryptophan hydroxylase activity in striate synaptosomes. Now, with the cofactor 2-amino-4-hydroxy-6-methyl-5-,6,7,8-tetrahydropteridine (6-MPH.; [10]), the sensitivity of our assay has increased [11] and we have measured the effects of short- and long-term administration of lithium chloride on solubilized synaptosomal enzyme from striate cortex, confirming that lithium induces and maintains a delayed increase in intrasynaptosomal tryptophan hydroxylase activity, countering the maintained stimulation of tryptophan uptake into synaptosomes [9,12] and returning the conversion measure to control values.

METHODS

Materials

L-[1-^{14}C]-Tryptophan (15 mCi/mmol) and L-[3-^{14}C] -Tryptophan (50 mCi/mmol) were purchased from New England Nuclear Corporation, Boston, Mass. L-[2,3-^{3}H]-tyrosine (12 Ci/mmol) was purchased from Amersham-Searle, Arlington Heights, Ill., and $DMPH_4$ and 6-MPH_4 were purhcased from Calbiochem, La Jolla, Ca. Adult male Sprague-Dawley rats (150 to 200 g each). obtained from Hilltop Lab Animals, Inc., Scottsdale, Pa., were individually caged and given free access to food and water. At specified times prior to sacrifice rats were given subcutaneous injections of various doses of lithium chloride (Mallinckrodt Chemical Works, St. Louis, Mo.) or sodium chloride crystals dissolved in water. Although daily doses of 5 to 10 meq of lithium chloride per kg are reportedly toxic to rats weighing 300 to 400 g [13], within the limits of our paradigm the smaller rats behaved normally and showed no neurological abnormalities.

Immediately after decapitation, brain regions were dissected freehand according to Craigie's Neuroanatomy of the Rat [14]. The striate cortex (wet weight, 80 mg) included most of the caudate and putamen and some globus pallidus, and the midbrain sample (130 mg extended rostrally up to the mamillary bodies.

Regional subcellular fractionation

Individual regions were homogenized in 25 volumes of ice-cold 0.32 M sucrose in a Thomas glass-Teflon homogenizer with clearance of 0.025 cm. Because of the limited amount of tissue from a single animal, in some experiments like regions were pooled from animals that had received identical treatment. After centrifugation at 1,000g the pellets (nuclei and cell debris) were discarded, and the supernates were centrifuged at 12,000g for 20 minutes [5]. After those supernates were transferred to clean tubes, the pellets were resuspended in the original homogenization volume of 0.32 M sucrose. Without delay, tryptophan hydroxylase activity was measured in the supernates and synaptosomal conversion of tryptophan to serotonin was measured in the pellets. In some experiments we homogenized (with a clearance of 0.01 cm) the midbrain and striate tissue in 0.01 M Tris-acetate buffer (pH 7.4) and centrifuged the homogenates at 35,000g for 20 minutes. Practically all the measurable enzyme activity in both regions appeared in the supernates. This hynotonic soluble preparation we distinguish from both solubilized synantosomal and isotonic soluble preparations, which are described below.

Our conversion measure requires maintenance of isotonicity to preserve the integrity of the synaptosomes. To measure the enzyme activity component of the conversion measure we resuspended the 12,000g pellet in 0.0001 M Tris-acetate buffer (pH 7.4) and centrifuged it at 35,000g for 30 minutes. The resulting pellet contained negligible enzymatic activity, and we measured the solubilized synaptosomal enzyme activity in the supernate in the presence of 6-MPH_4.

To measure the pharmacological effects of lithium chloride on tryptophan hydroxylase activity, we assayed the

soluble fraction from midbrain and the solubilized synaptosomal fraction from striate cortex, in view of observations that soluble enzyme activity is correlated with serotonergic cell body regions and synaptosomal conversion activity is correlated with serotonergic nerve ending regions [11,16]. We also measured synaptosomal conversion of tryptophan to serotonin in striate tissue homogenized in 0.32 M sucrose and fractionated as described above.

The assays

The assay for tryptophan hydroxylase developed by Ichiyama et al. [17,18] couples tryptophan hydroxylase with aromatic amino acid decarboxylase (EC 4.1.1.26) and is possible because the decarboxylase manifests distinctly different affinities for tryptophan (K_m = 1 mM) and 5-hydroxytryptophan (K_m = 20 µM). L-[1-^{14}C]-tryptophan was dissolved in 0.1 M Tris-acetate buffer (pH 7.4], and impurities were removed by lyophilization. Our optimal incubation mixture contained 40 µmol Tris-acetate buffer (pH 7.4), 400 nmol 6-MPH_4, 700 nmol dithiothreitol, 5 to 10 units of aromatic L-amino acid decarboxylase from rat kidney, 100 to 200 µ1 enzyme preparation (0.3 to 0.6 mg protein, and 4 to 6 nmol L-[1-^{14}C]-tryptophan (15 mCi/mmol) in a final volume of 700 µ1. Our preparation of decarboxylase from rat kidney [9] was a modification of the method of Christenson et al. [19]. In this assay the highest usable substrate concentration is 10 µM, and in the presence of 6-MPH_4 rather than $DMPH_4$ measurable enzyme activity increased more than eight fold; the enzyme's affinity for cofactor doubled; and its affinity for substrate more than tripled [10,11].

In the assay for conversion of tryptophan to serotonin the optimal incubation mixture contained 40 umol Tris-acetate buffer (pH 8.1), 100 to 200 µ1 enzyme preparation (0.1 to 0.3 mg protein), and 4 to nmol L-[1-^{14}C]-tryptophan (15 mCi/mmol) in a final volume of 700 µl.

For either assay, blanks contained boiled enzyme or the appropriate buffer. Before incubation the mixtures were sealed in 15-ml tubes with rubber caps from which were suspended plastic wells (Kontes Glass Company, Vineland, N.J.) containing 100 µl of NCS (Nuclear-Chicago Corporation, Des Plaines, Ill.) for the collection of $14CO_2$. All samples were incubated with shaking for 45 minutes at 37° C, and the reactions were stopped by the injection of 500 µl of 2 N perchloric acid through the caps with an automatic syringe. The $^{14}CO_2$ accumulated in the NCS over three more hours of incubation at 37° C in the shaker. The wells were then removed and placed directly into counting vials containing 10 ml of a mixture of toluene phosphor (80 ml toluene containing 3.4 g PPO and 0.41 g POPOP) and absolute ethanol in a ratio of 4 to 1. Radioactivity was counted in a Beckman LS-250 liquid scintillation spectrophotometer with external standard quench correction.

The assays for both tryptophan hydroxylase activity and synaptosomal conversion were linear with time for as long as 45 minutes and with protein over the range of protein concentration examined. Products of the conversion assay were analyzed by TLC [ll].

Synaptosomal uptake of tryptophan

Aliquots of the pellets from which samples to measure conversion were taken served as the tissue source for studies of the effect of lithium chloride on substrate uptake. The incubation medium was Krebs-Ringer phosphate buffer containing 100 mM NaCl, 5 mM KCl, 2 mM KH_2PO_4 , 2 mM $MgSO_4$ $7H_20$, 3.1 mM $NaHC0_3$, 1.5 mM $NaPO_4$ buffer (0.1 M, pH 7), 5.5 mM sodium fumarate, 5 mM sodium pyruvate, 5 mM sodium glutamate, and 12 mM D-glucose [20]. In total volumes of 600 to 700 µ1 there were 0.2 to 0.3 mg protein, and substrate concentrations were varied from 10 to 53 µM, within the range of the high affinity uptake system for tryptophan [21]. Uptake was stopped by dilution with 0.32 M sucrose, and the synaptosomes were collected on Millipore filters (25 mm diameter, 0.65 µ pores) with a Millipore multiple-sampling manifold. Each filter was washed immediately with 2 ml of 0.32 M sucrose, and then radioactivity was counted in 10 ml of toluene phosphor/ethanol in a Beckman LS-200 liquid scintillation counting system. The uptake of L-[3-^{14}C]-tryptophan (50 mCi/mmol) was linear with time for 4 minutes at 37° C and with protein concentration from 0.06 to 0.4 mg.

Synaptosomal uptake of tyrosine

For comparison we measured the effects of lithium chloride on the uptake of radioactive tyrosine in tissue from the same source. The incubation constituents were the same except for substrate, which varied in concentration from 8 to 42 µM. Incubations proceeded and radioactivity was measured as above, except that the counting solution was 10 ml Aquasol (New England Nuclear, Boston, Mass.). Synaptosomal uptake of L-[^{3}H]-tyrosine was linear for 4 minutes at 37° C.

RESULTS AND DISCUSSION

Stimulation of high affinity tryptophan uptake

Administration of lithium chloride (5 to 10 meq/kg/day) stimulated the uptake of radioactive tryptophan by striate synaptosomes, the latency of the stimulation varying with the dose. With 10 meq the V_{max} of the uptake reaction was increased within three days (Figure 1). With 5 meq there were no changes by the third day, but at 21 days the V_{max} was augmented. Although augmentation of V_{max} reflects conditions consistent with an increase in the uptake of radioactive tryptophan, the Km for the uptake was either unchanged or increased only slightly from control values.

FIGURE 1

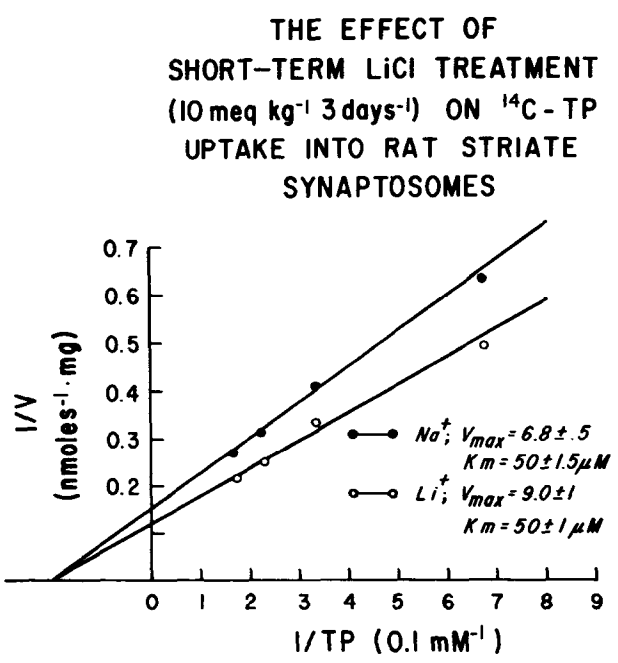


Figure 1: Velocity is plotted as nmoles of L-(3-^{14}C)-tryptophan retained per mg per 4 minutes. Substrate concentrations ranged from 15 to 53 µM. V_{maz} and K_m ± SE are estimated according to the method of Wilkinson (44).

Our index of uptake is the radioactivity retained after incubation of the synaptosomes with radioactive substrate. However, lithium treatment has reportedly inhibited catecholamine release from nerve endings [5]. so, to rule out both similar inhibition of serotonergic release and facilitated exchange of endogenous for exogenous tryptophan, we preincubated synaptosomes from rats treated with lithium chloride or sodium chloride for 5 minutes with L-[3-^{14}C]-tryptophan (10 µM) and found that over 10 minutes the efflux of radioactivity from the preparations from lithium-treated rats was essentially the same as that from sodium-treated rats.

Stimulation of the uptake of radioactive tryptophan by striate synaptosomes in vitro is relatively specific when compared to the effect on the uptake of radioactive tyrosine, and also relatively dependent on the level of lithium present. When synaptosomes were incubated with 0 to 50 mM lithium chloride or sodium chloride, high affinity uptake of radioactive tryptophan was stimulated by 10 mM lithium chloride and stimulated more by 50 mM lithium chloride. The of the reaction did not change, but the V_{max} rose 45 percent with 50 mM lithium chloride. The K_m for the uptake of radioactive tyrosine was appreciably higher than that for radioactive tryptophan under control conditions at equimolar concentrations of substrate (50 µM versus 200 µM) but stimulation of the uptake of radioactive tyrosine in the presence of 50 mM lithium chloride was only 20 percent (versus 45 percent for tryptophan). We chose these relatively high absolute levels of lithium for their efficiency in vitro and did not try to reflect possible mechanisms in vivo where dose and time interact.

Stimulation of conversion of tryptophan to serotonin

Both time and dose are related to the effects of lithium chloride on the conversion of radioactive tryptophan to

serotonin by striate synaptosomes (Figure 2). High doses of lithium in *in vivo* resulted in 30 to 40 percent increases in conversion as well as uptake (Figures 1 and 2). Serum levels

FIGURE 2

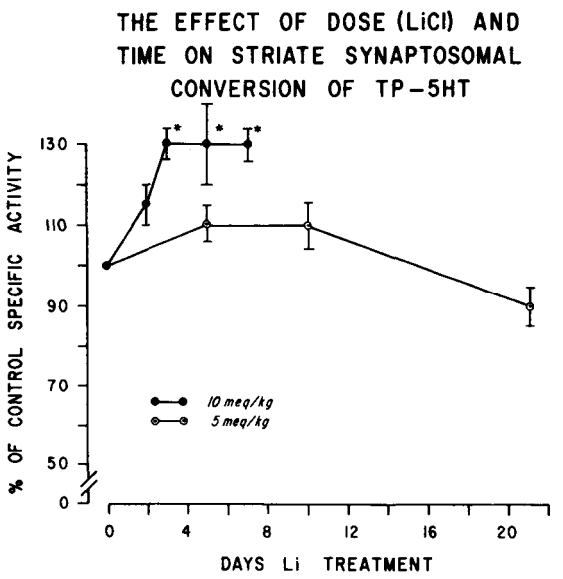


*Figure 2: Controls received equal doses of NaCl. Animals were sacrificed 24 hours after the final daily subcutaneous drug administration. Control activity: 200 pmoles per mg per 45 minutes. * p<0.01.*

of lithium in the rats at the time of sacrifice ranged from 0.8 to 1.0 meq per liter [22]. *In vitro* much higher levels of lithium were required to produce comparable increments in either uptake or conversion. At substrate concentrations of about 10 µM and identical doses of lithium chloride, the specific activity of conversion was between 5 and 7 pmol product per mg protein per minute, while that of high affinity uptake was between 350 and 450 pmol. In spite of the large disparity in the absolute values, we have elsewhere demonstrated similar proportional changes in conversion and uptake with other treatments [9,11,21]. Such a phenomenon might be explainable by the presence of a preferential pathway for uptake and synthesis, a high affinity uptake for substrate in cells other than serotonergic neurons [23,241, or a relatively low net hydroxylation because of the efflux of unconverted tryptophan.

Effects on tryptophan hydroxylase activity in the midbrain

The cell bodies of the serotonergic system are in the raphé nuclei of the brain stem (B7, B8, and B9; [25]), a region dominated by soluble tryptophan hydroxylase [12,16,24]. From homogenization of midbrain tissue in 0.32 M sucrose and centrifugation at 12,000g for 20 minutes, we obtain an isotonic soluble preparation, which is distinct from the hypotonic soluble and solubilized synaptosomal preparations characterized above. With 10 meq/kg of lithium chloride a progressive decrement in midbrain isotonic soluble tryptophan hydroxylase activity began on the first day (Figure 3). A maximum

FIGURE 3

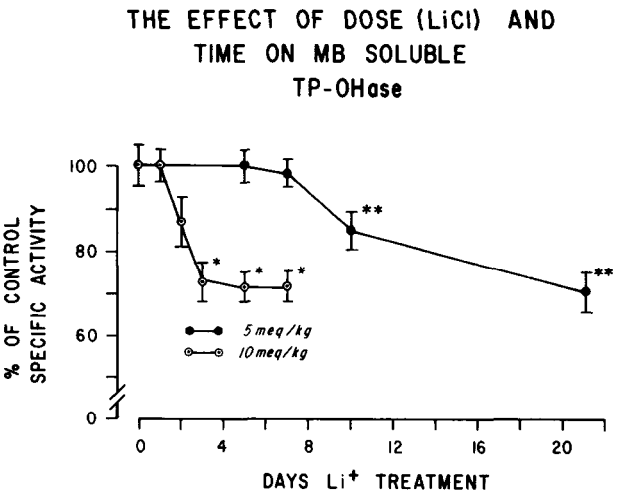


*Figure 3: Controls received equal doses of NaCl. Animals were sacrificed 24 hours after the final daily subcutaneous drug administration. Control activity: 500 ± 15 pmoles per mg per 45 minutes. * p<0.002; ** p<0.01.*

decrement of about 30 percent was reached on the third day, and was still present on the fifth and seventh days. With a lower dose (5 meq/kg) ten days of drug administration were required for a significant decrement to appear, although the maximum decrement with the lower dose also reached 30 percent. As with uptake and conversion activities (Figures 1 and 2), interactions between dose and time are implicit in the

effects of lithium treatment on soluble tryptophan hydroxylase activity (Figure 3), although, in vitro, concentrations of drug from 1 to 100 mM had no effect on the activity of midbrain isotonic soluble enzyme activity.

The decrease in enzyme activity observed after treatment with lithium chloride could be due to a decrement in the number of available enzyme sites or even molecules because the kinetics of the reduced *enzyme* activity with respect to cofactor showed a decrease ln V_{max} rather than a change in K_m, and the decrement appears to move by fast axonal flow from the raphé nuclei to the striate cortex. It is not clear whether the change induced by lithium chloride might be secondary to decreased synthesis or increased turnover of tryptophan hydroxylase.

Simultaneous measurement of tryptophan hydrozylase activity and synaptosomal conversion of tryptophan to serotonin

We monitored simultaneously the effects of short-term daily administration of lithium chloride (10 meq/kg) on (1) striate synaptosomal uptake of radioactive tryptophan, (2) conversion of tryptophan to serotonin by striate synaptosomes, (3) midbrain tryptophan hydroxylase activity, and (4) solubilized striate synaptosomal tryptophan hydroxylase activity (Figure 4). Both the uptake and the conversion of radioactive tryptophan to serotonin began to rise within the first day of treatment. Soluble enzyme activity from the serotonergic cell bodies in the midbrain and from the lysed striate synaptosomes showed no change the first day. On the second day the increases in uptake and conversion did not continue, but the enzyme activity from the cell bodies and nerve endings began to decrease. On the third day the uptake of substrate and its conversion to transmitter increased further. After 21 daily treatments with lithium chloride (5 meq/kg) the decrement in cell body and nerve ending enzyme activity was still present, as was the increase in the uptake of radioactive tryptophan; conversion of radioactive tryptophan to serotonin had returned to control levels (Figure 5). As we speculated before [9,12], the values for conversion after 21 days of treatment seem to represent the net effect of the lithium-stimulated increase in uptake (and conversion) and a decrease in intraneuronal rate-limiting biosynthetic enzyme activity.

FIGURE 4

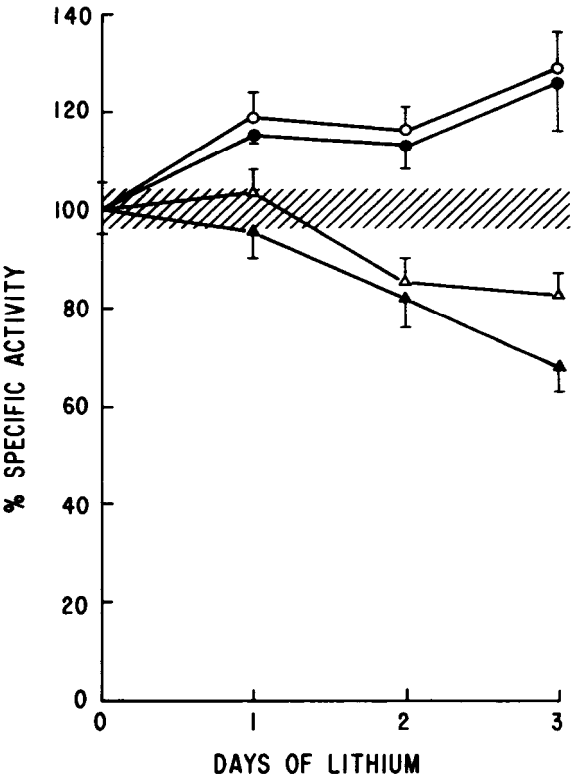


Figure 4: Simultaneous effects of 3 daily lithium injections (10 meq/kg) on four measures related to serotonin synthesis. Control velocities: o-o high affinity uptake of (^{14}C)-tryptophan (10μM),-1220 ± 50.pmol/mg/4 min; ●—● striate synaptosomal conversion activity (8μM substrate), 250 ± 12 pmol/mg/45 min;Δ—Δ midbrain soluble enayme activity (8μM substrate), 525 ± 20 pmol/mg/45 min; ▲—▲ striate sotubilized enzyme activity (8μM substrate), 150 ± 5 pmol/mg/45 min. Each day uptake and conversion activities were increased ($p<0.05$). At 2 and 3 days the enayme activities were decreased ($p<0.05$).

FIGURE 5

THE EFFECT OF LONG TERM (3WKS) LITHIUM TREATMENT
ON RAT BRAIN SEROTONERGIC SYSTEMS

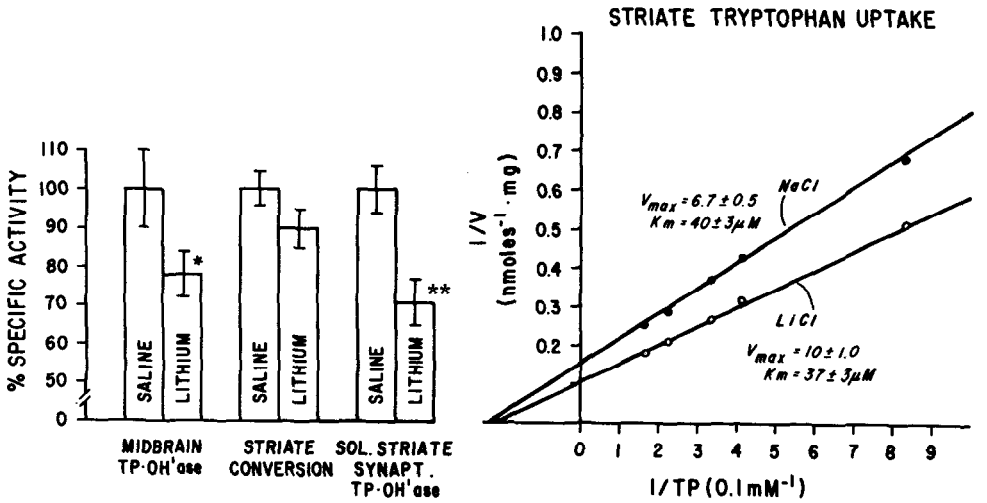


Figure 5: Simultaneous effects of 21 daily lithium injections (5 meq/kg) on four measures related to serotonin synthesis. Control velocities: midbrain soluble enzyme activity, 475 ± 20 pmol/mg/45 min (p<0.05; striate synaptosomal conversion activity, 200 ± 10 pmol/mg/45 min; striate solubilized enzyme activity, 160 ± 5 pmol/mg/45 min (** p<0.001). High affinity uptake velocity (12 to 53 μM substrate) is plotted as nmoles of L-(3-^{14}C)-tryptophan retained per mg per 4 minutes. V_{max} and K_m ± S.E. are estimated according to the method of Wilkinson (44).*

Stimulation of conversion of radioactive tryptophan to serotonin after tryptophan loads

Perez-Cruet el al. [26] have shown that treating rats with lithium carbonate increases serum and brain levels of tryptophan. To rule out increased extraneuronal tryptophan as an intervening variable in our experiments, we dissolved progressively larger amounts of L-tryptophan hydrochloride in saline and administered it to rats subcutaneously, and then determined the effects on conversion activity. Within the range of tryptophan loads tested the dose-response relationship was linear. The addition of cold tryptophan would, if anything, dilute the radioactivity of the product, so our results may even underestimate the increase in conversion. To determine whether a load-induced increase in uptake of radioactive substrate preceded the load-induced increase in conversion, we preincubated striate synaptosomes with 100 μM L-tryptophan and examined the uptake kinetics. The V_{max} increased, but the K_m for tryptophan did not, which suggests that the tryptophan load increased the uptake of radioactive tryptophan by increasing the number of uptake sites. We cannot tell whether this substrate-induced increase in uptake (and conversion) accounts for the initial effects of lithium because the concentration of tryptophan in vivo is obviously critical, and we do not know whether tryptophan loads can bring substrate levels within the appropriate range at the relevant sites. Attempts to determine regional and subcellular levels of tryptophan after the administration of exogenous tryptophan [26-28] are not conclusive.

SIGNIFICANCE

One of the intriguing aspects of psychotropic drug action is latency to action. In the case of lithium, treatment for mania may require five to 10 days to reach efficacy, and prophylaxis for both mania and depression may not achieve full

effectiveness for months or longer than a year [29-32]. The most conservative explanation of such temporal parameters is that the doses used to initiate treatment do not readily achieve effective levels in the blood or brain. Schou [33-35] and others have reported that in animals brain levels of lithium do not achieve a steady state for the first five to seven days of daily drug administration. However, Schou (personal communication) has also found that large initial doses of lithium reduce the latency to action very little while magnifying the clinical side effects greatly. Those studies and our own clinical experience lead us to believe that plasma or brain blood levels, while perhaps accounting for some latency to effectiveness, do not account for it all. When we consider the sequence of events observed in the laboratory over a human time base, the possibility arises that some aspects of clinical efficacy may be due to the secondary or tertiary adaptive mechanisms set in motion by the primary drug effects [36]. Generally, conventional "tolerance" fails to develop to psychotherapeutic drugs, although the many so-called side effects do diminish or disappear with chronic administration. Nonetheless, the prophylactic and antimanic effects of lithium (as well as the antipsychotic effects of the neuroleptics and the antidepressant effects of the thymoleptics) do not usually require progressively greater doses; the same dose may actually become more efficacious over time. Moreover, these treatments tend to retain their clinical efficacy over months, years, and even decades. It might be said that tolerance mechanisms induced by such drugs as morphine, alcohol, or amphetamine simply overcome their primary actions very quickly [21,37,38]. Furthermore, we suggest that treatment with lithium (as well as neuroleptics and thymoleptics [39]) could be de facto, treatment by the induction of tolerance mechanisms.

The almost perfect symmetry of lithium prophylaxis against both manic and depressive components of affective disorder must be the keystone in any construction of a neurobiological model of the drug's action. For example, to explain the antimanic effects of lithium by saying that it inhibits the release of norepinephrine, even if that were proved to be true, would be inadequate for two reasons. Our recent study [40] has shown that tolerance develops within eight days to lithium-induced suppression of amphetamine-induced hyperactivity, and that it is associated with increases in tyrosine hydroxylase activity in the nigro-striatal pathway. Secondly, what would be said about its antidepressant effects? The sequence of events induced in central serotonergic biosynthetic nmechanisms by the chronic administration of lithium hints at adaptive systems capable of increasing serotonin svnthesis as well as decreasing it: The relationships among serotonin synthesis, levels, turnover, and metabolism and behavioral states in animals and man are extremely complex, and it is possible that serotonin serves a regulatory role rather than a direct role with regard to behavior. For example, tryptophan has been administered in conjunction with both families of antidepressant medication, thymoleptics and monoamine oxidase inhibitors, with varying degrees of success and failure. There have been suggestions that 5-hydroxytryptophan may be antimanic, and brain stem 5-hydroxyindoleacetic acid has been found to be low in patients who committed suicide, yet Coppen and his coworkers [41] have found low 5-hydroxyindoleacetic acid in the CSF of patients with affective disorder when they were sick or well. Although no tidy hypothesis relating increases or decreases in serotonin synthesis to one or another pole of the affective continuum is possible at present, our findings do suggest another possibility.

The control levels of nerve ending serotonin synthesizing capacity after chronic lithium treatment represent the net effect of two maintained changes that were induced by the drug, i.e. the increase in the high affinity uptake of tryptophan and the decrease in intrasynaptosomal rate-limiting enzyme activity. Although each of these changes can be induced separately by other agents--tryptophan loads activate substrate transport on a dose-response basis, and amphetamine decreases intraneuronal tryptophan hydroxylase--neither the increased uptake nor the decreased intrasynaptosomal enzyme activity can be extended after lithium treatment. It is as though the uptake were elevated and stabilized at a ceiling, the intraneuronal enzyme activity were reduced and stabilized at the floor, and the bi-directional

adaptive capacity were "used up," having returned the overall measure Of nerve ending biosynthetic capacity, the conversion rate, to baseline.

Perhaps lithium's efficacy in preventing or dampening both manic and depressive episodes without changing many control-level or normal behaviors in the intersymptomatic intervals is related to the way lithium influences the serotonergic system. It was gratifying to learn of an almost point for point correlation between CSF metabolites in man and this sequence of neurobiological events in rats. Goodwin et al. [42] report a biphasic response in 5-hydroxyindoleacetic acid turnover in the CSF of depressive patients after five and 21 days of clinical doses of lithium. After probenecid blockade of metabolite egress from the CSF, 5-hydroxyindole-acetic acid was elevated after five days of lithium carbonate (corresponding to the increase in tryptophan uptake and conversion to serotonin by synaptosomes) and then back to or below control level after 21 days (corresponding to the state of affairs we illustrate in Figure 5).

An implication of this model for the action lithium in treatment is the possibility that daily or regular tryptophan loads might be prophylactic in bipolar affective disorder. The increased circulating tryptophan might increase uptake of substrate into the nerve endings by mass action rather than stimulation of the uptake mechanism, as with lithium, leading to increased transmitter synthesis and a reflexive decrease in tryptophan hydroxylase, which would return nerve ending conversion of tryptophan to serotonin to normal. Thus uptake and conversion might be pushed to their ceiling and tryptophan hydroxylase activity pushed to the floor, narrowing the range of potential adaptive changes without changing the control level of function. Recently it has been reported that daily tryptophan loads can substitute for lithium as effective prophylaxis in affective disease in patients who show marked intolerance to lithium (N. Kline, personal communi-cation). Murphy et al. [43] have recently reported both antidepressant and antimanic effects from tryptophan given to patients with bipolar disorder.

Much more work needs to be done at basic and clinical levels before it will be clear whether the clinical action of lithium can, in fact, be explained by this kind of "cross-tolerance" for psychopatholog-ical amounts of neurotransmitter. For now, it may serve heuristically to help us explore the complex adaptive capacity of the serotonergic system in brain.

ACKNOWLEDGMENT

This work is supported by United States Public Health Service Grants MH-14360, DA-00046, and DA-00265.

REFERENCES

1. Angst, J., P. Weis, P. Grof, P. Baastrup and M. Schou. Lithium prophylaxis in recurrent affective disorders. Br. J. Psychiat. 116: 604-614, 1970.

2. Coppen, A., R. Noguera, J. Bailey et al. Prophylactic lithium . affective disorders. Lancet 1971-II: 275-279, 1971.

3. Skou, J.C., Enzymatic aspects of active linked transport of Na^+ and K^+ through the cell membrane. Prog. Biophys. Mol. Biol. 14: 133-166, 1964.

4. Whittam, R. and M.E. Ager. Vectorial aspects of adenosine-triphosphatase activity in erythrocyte membranes. Biochem. J. 93: 337-348, 1964.

5. Katz, R.I., T.N. Chase and I.J. Kopin. Evoked release of norepi-nephrine and serotonin from brain slices: inhibition by lithium. Science 162: 466-467, 1968.

6. Corrodi, H., K. Fuxe and M. Schou. The effect of prolonged lithium administration on cerebral monoamine

neurons in the rat. Life Sci. 81: 643-651, 1969.

7. Sheard, M.H. and G.K. Aghajanian. Neuronally activated metabolism of brain serotonin: effect of lithium. Life Sci. 911: 285-290, 1970.

8. Ho, A.K.S., H.H. Loh, F. Craves, R.J. Hitzemann and S. Gershon. The effect of prolonged lithium treatment on the synthesis rate and turnover of monoamines in brain regions of rats. Eur. J. Pharmacol. 10: 72-78, 1970.

9. Knapp, S. and A.J. Mandell. Short-and long-term lithium administration: effects on the brain's serotonergic systems. Science 180: 645-647, 1973.

10. Friedman, P.A., A.H. Kappelman and S. Kaufman. Partial purification and characterization of tryptophan hydroxylase. Biol. Chem. 247: 4165-4173, 1972.

11. Knapp, S., A.J. Mandell and M.A. Geyer. Effects of amohetamines on regional tryptophan hydroxylase activity and synaptosomal conversion of tryptophan to 5-hydroxytryptamine in rat brain. J. Pharmacol. Exp. Ther. 189: 676-689, 1974.

12. Knapp, S. and A.J. Mandell. Effects of lithium chloride parameters of biosynthetic capacity for 5-hydroxytryptamine in rat brain. J. Pharmacol. Exp. Ther. 193: 812-823, 1975.

13. Schildkraut, J.J. Pharmacology--the effects of lithium on biogenic amines. In: Lithium: Its Role in Psychiatric Research and Treatment edited by S. Gershon and B. Shopsin, Plenum Press, New York, 1973.

14. Zeman, W. and J.R.M. Innes. Craigie's Neuroanatomy of the Rat, Academic Press, New York, 1963.

15. Gray, E.G. and V.P. Whittaker. The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. J. Anat. 96: 71-88, 1962.

16. Knapp, S. and A.J. Mandell. Parachlorophenylalanine: its three phase sequence of interactions with the two forms of brain tryptophan hydroxylase. Life Sci. 11I: 761-771, 1972.

17. Ichiyama, A., S. Nakamura, Y. Nishizuka and O. Hayaishi. Tryptophan hydroxylase in mammalian brain. Adv. Pharmacol. 6A: 5-17, 1968.

18. Ichiyama, A., S. Nakamura, Y. Nishizuka and O. Hayaishi. Enzymic studies on the biosynthesis of serotonin in mammalian brain. J. Biol. Chem. 245: 1699-1709, 1970.

19. Christenson, J.G., W. Dairman and S. Udenfriend. Preparation and properties of a homogeneous aromatic L-amino acid decarboxvlase from hog kidney. Arch. Biochem. Biophys. 141: 356-367, 1970.

20. Dawson, R.M.C., D.C. Elliott, W.H. Elliott and K.M. Jones, editors. Data for Biochemical Research, Oxford University Press, Cambridge, 1969.

21. Knapp, S. and A.J. Mandell. Narcotic drugs: effects on serotonin biosynthetic systems of the brain. Science 177: 1209-1211, 1972.

22. Zettner, A., K. Rafferty and H. Jarecki. Determination of lithium in serum and urine by atomic absorption spectroscopy. Atomic Absorption Newsletter 7: 32-34, 1968.

23. Kuhar, M.J., R.H. Roth and G.K. Aghajanian. Synaptosomes from forebrains of rats with midbrain raphe lesions: selective reduction of serotonin uptake. J. Pharmacol. Exp. Ther. 181: 36-45, 1972.

24. Mandell, A.J., S. Knapp and L.L. Hsu. Some factors in the regulation of central serotonergic synapses. Life Sci. 14: 1-17, 1974.

25. Dahlstrom, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system. Acta Physiol. Scand. 62: suppl. 232: 1-55, 1964.

26. Perez-Cruet, J., A. Tagliamonte, P. Tagliamonte and G.L. Gessa. Stimulation of serotonin synthesis by lithium. Pharmacol. Exp. Ther. 178: 325-330, 1971.

27. Fernstrom, J.D. and R.J. Wurtman. Brain serotonin content: physiological dependence on plasma tryptophan levels. Science 149: 149-152, 1971.

28. Grahame-Smith, D.G. Studies in vivo on the relationship between brain tryptophan, brain 5-HT synthesis, and

hyperactivity in rats treated with a monoamine exidase inhibitor and L-tryptophan. J. Neurochem. 18: 1053-1066, 1971.

29. Cade, J.F.J. Lithium salts in the treatment of psychotic excitement. Med. J. Australia 36: 349-352, 1949.

30. Schou, M., N. Juel-Nielsen, E. Stromgen and H. Volby. The treatment of manic psychoses by the administration of lithium salts. J. Neurol. Psychiat. 17: 250-260, 1954.

31. Baastrup, P.C. and M. Schou. Lithium as a prophylatic agent: its effect against recurrent depressions and manic-depressive psychosis. Arch. Gen. Psychiat. 16: 162-172, 1967.

32. Freyhan, F.A. Lithium treatment: prophylactic or compensatory? Am. J. Psychiat. 128: 122, 1971.

33. Schou, M. Biology and pharmacology of the lithium ion. Pharmacol. Rev. 9: 17-58, 1957.

34. Schou, M. Possible mechanisms of action of lithium salts: approaches and perspectives. Biochem. Soc. Trans. 1: 81-87, 1973.

35. Schou, M. Prophylactic lithium maintenance treatment in recurrent endogenous affective disorders. In: Lithium: Its Role in Psychiatric Research and Treatment, edited by S. Gershon and B. Shopsin, Plenum Press, New York, 1973.

36. Mandell, A.J. Neurobiological mechanisms of presynaptic metabolic adaption and their organization: implications for a pathophysiology of the affective disorders. In: Neurobiological Mechanisms of Adaption and Behavior, edited by A.J. Mandell, Raven Press, New York 1975.

37. Mandell, A.J., D. Segal, R. Kuczenski and S. Knapp. Some macromolecular mechanisms in CNS neurotransmitter pharmacology and their psychobiological organization. In: Chemistrv of Mood, Motivation, and Memory, edited by J. McGaugh, Plenum Press, New York, 1972.

38. Rogawski, M.A., S. Knapp and A.J. Mandell. Effects of ethanol on tryptophan hydroxylase activity from striate synaptosomes. Biochem. Pharmacol. 23: 1955-1962, 1974.

39. Segal, D.S., R. Kuczenski and A.J. Mandell. Theoretical implications of drug-induced adaptive regulation for a biogenic amine hypothesis of affective disorder. Biol. Psychiat. 9: 147-159, 1974.

40. Segal, D.S., M. Callaghan and A.J. Mandell. Alterations in behavior and catecholamine synthesis induced by lithium. Nature 254: 58-59, 1975.

41. Coppen, A.J. The chemical pathology of the affective disorders. Sci. Basis Med. Ann. Rev. 179-198, 1970.

42. Goodwin, F.K., R.L. Sack and R.M. Post. Clinical evidence for neurotransmitter adaption in response to antidepressant therapy. In: Neurobiological Mechanisms of Adaption and Behavior, edited by A.J. Mandell, Raven Press, New York, 1975.

43. Murphy, D.L., M. Baker, F.K. Goodwin. H. Miller. J. Kotin and W.E. Bunney, Jr. L-Tryptophan in affective disorders: indoleamine changes and differential clinical effects. Psychopharmacologia 34: 11-20, 1974.

44. Wilkinson, G.N. Statistical estimations in enzyme kinetics. Biochem. J. 80:324-332, 1961.

AUTHORS

Arnold J. Mandell, Ph.D., and Suzanne Knapp, Ph.D.: Department of Psychiatry, School of Medicine, University of California, San Diego, La Jolla, California, 92037.

EFFECTS OF DISULFIRAM ON THE AMPHETAMINE-INDUCED BEHAVIORAL SYNDROME IN THE CAT AS A MODEL OF PSYCHOSIS

A. Sudilovsky

INTRODUCTION

The use of psychoactive drugs as experimental tools holds a fascination for those interested in psychiatric research because it offers possibilities for a better understanding of the correlations between the psychopathological manifestations of functional psychoses and their underlying mechanisms. Consequently, an effort has been made in the past decades to alter pharmacologically the behavior of animals or humans in the direction of psychosis. Focus on the mechanisms of action of psychotogenic and psychotropic compounds has produced convergent lines of evidence that the biogenic amines of the brain are involved in the modulation of disturbed behavior. Furthermore, in recent years a "dopamine hypothesis" of schizophrenia, i.e. central dopaminergic overactivity, has been advanced; it seems useful, as a frame of reference, therefore, to provide here its general outline.

The administration to experimental animals of amphetamine*, a sympathomimetic amine that potentiates the effect of catecholamines, results in the stimulation of certain psychomotor components of behavior and the concurrent reduction or suppression of others (70,72,78). Typically, a decrease in locomotion and the development of a hyperaroused hyperkinetic state, including species-specific stereotyped compulsive motor activity, and the abolition of ordinary goal-seeking motivated behavior are observed after single or repeated administration of moderate doses of the drug (73). The stereotyped activity is a conspicuous and reproducible phenomenon apparently obtainable in all mammals and is currently used 'in the preclinical screening of new compounds for potential antipsychotic properties (50). Blockade of stereotypy has highly predictive value, and has been helpful to detect many of the effective antischizophrenic drugs now available (22). There exists extensive evidence from pharmacologic, neuroanatomic ablation, and micro-injection studies that dopamine plays an important role in the mediation of amphetamine-induced stereotypy (36,72,73). Consistent with this evidence is the 'finding of a lack of stereospecificity for the d and l isomers of amphetamine, both in inhibiting catecholamine uptake in

**Throughout this paper the expressions 'amphetamine' and 'methamphetamine' are used interchangeably.*

synaptosomes of dopamine-containing areas of the rat brain (striatum) and in inducing stereotyped behavior, as contrasted with a tenfold stereospecificity for the d isomer in inhibiting catecholamine uptake in synaptosomes of norepinephrine-containing areas and in enhancing locomotor activity (89).

In human subjects, amphetamine elicits stereotyped behavior comparable to that seen in animals (29,73,76), but may also lead to the occurrence of psychotic episodes closely resembling paranoid schizophrenia (6,18,41) irrespective of the pre-existence of the disease or of schizoid tendencies (6,7,10). The results of experiments in animals (28,72,73) as well as of those in humans (7,10) suggest the involvement of dopamine in the elicitation of this stimulant psychosis. For example, comparison of the dose-response relationships of d and 1 amphetamine in producing psychosis has shown their relative potencies to vary from 1:1 to 2:1, making it im- probable that norepinephrine events play a crucial role and suggesting that dopaminergic or other, as yet unknown, non-stereospecific mechanisms are critical. These results, as well as the frequent misdiagnosis of amphetamine psychosis as paranoid schizophrenia (13,18,52), the specific activation of schizophrenic symptoms by small doses of the drug (20,48), and the effectiveness of antischizophrenic agents in the treatment of amphetamine intoxication (30) and psychosis (8), argue strongly for a relationship between the mechanisms underlying amphetamine stereotypy and psychosis and those underlying certain forms of schizophrenia.

On the other hand, there is also evidence that overactivity in the dopaminergic system or some of its pathways might contribute to the pathogensis of schizophrenia: the high degree of correlation between the therapeutic activity of neuroleptics in man and both the antistereotypy effect and the blocking activity of such compounds on dopamine receptors (45,49,75) or on the presynaptic impulse-coupled release of dopamine (79). Such a notion is consistent with the lack of therapeutic activity of agents that are structurally related to the neuroleptics but do not block dopamine receptors (64), the ability of antipsychotic agents to evoke Parkinsonian side effects that are regarded as manifestations of reduced dopaminergic activity in the nigrostriatal system (47), the worsening of schizophrenics or the occurrence of psychotic reactions in Parkinsonian patients after the administration of the dopamine precursor L-Dopa (9), the infrequent occurrence of schizophrenia in patients suffering from Parkinson's disease (which is characterized by low concentrations of dopamine in the nigro-striato-pallidal system), and the amelioration of psychotic symptomatology in schizophrenics affected with encephalitis lethargica (75). Further support for this line of reasoning lies in the recent demonstration of dopamine nerve terminals in the limbic cortex of the rat (441, since a cortical dopamine innervation in man (still unproved could be related to certain symptoms in schizophrenia, i.e., thought disorders, impairment of goal-directed behavior, disturbances of affect, and hallucinations, the implied cortical dysfunction occurring in addition to the involvement of subcortical areas.

A number of excellent reviews of work on catecholamine neuronal pathways, as well as on other significant findings that either support or weaken the dopamine hypothesis, can be found in the literature (20,25,29,34,56,62,64, 75,82). The indirect and inferential nature of the evidence, which does not preclude the participation of other neurotransmitter systems, has permitted invoking a multiplicity of mechanisms as the basic alteration. These postulated mechanisms include exaggerated release of the transmitter, supersensitivity of the receptor, defective feedback control of dopamine pathways, imbalance of the interaction with other neurotransmitter systems, i.e. cholinergic, noradrenergic, serotonergic, or GABAergic, central overload of dopamine from decreased conversion to norepinephrine, or formation of abnormal psychotogenic metabolites. In addition, at the anatomical level, hyperinnervation of dopamine areas or plastic compensatory growth of dopamine terminals into areas previously devoid of them, as a result of a primary lesion in other pathways, should be considered.

In light of the above, the relevance of the amphetamine intoxication process for psychiatric research becomes apparent; indeed, of all drugs amenable to use in the production of a model

psychosis, amphetamine is now recognized as the most valuable (22,80). During the past several years, we have stressed the need for employing direct observational techniques in the assessment of animal behavior (28,84) This approach permits the evaluation of the entire behavioral spectrum, including qualitative as well as quantitative aspects, and might lend itself to the establishment of correlates of human symptomatology, thereby adding a psychopathologically meaningful dimension to the paradigm.

We have previously reported that, from a phenomenological standpoint, the behavioral manifestations of cats chronically intoxicated with amphetamine parallel the evolution of the paranoid psychosis induced by the drug in humans (29). However, a group of manifestations in the cat, such as frozen postures, disjunctive behaviors and postures, cataleptoid phenomena, obstinate progression, and loss of righting reflex, did not appear to be consistent with the phenomenology of the paranoid psychosis.

Since treatment of schizophrenic patients with disulfiram (tetraethylthiuram disulfide), an inhibitor of norepinephrine synthesis that acts at the level of the enzyme dopamine beta-hydroxylase thereby leading to increased dopamine concentrations, had been found to profoundly exaggerate psychotic symptomatology (43), the current experiments were undertaken to examine the amphetamine behavioral syndrome in the cat as it is modified by pretreatment with disulfiram. It was hypothesized that the end-stage behaviors characteristic of chronic amphetamine intoxication would develop early in the animals pretreated with disulfiram, and that a more critical differentiation of psychomotor alterations relevant to the model psychosis could be obtained through possible activation of given behaviors. If a differential enhancement of certain aspects of behavior occurred, it might then be assumed that such aspects bear a relationship to a stimulated dopamine system in the relative absence of norepinephrine action, whereas those not affected or affected only later are based on other mechanisms.

METHODS

Twenty-six female cats, 2.5 to 3.5 kg, were used. All animals were housed in individual cages and fed ad libitum throughout the experiment. Under sodium pentobarbital anesthesia (36 mg/kg i.p.), stainless steel electrodes were implanted stereotaxically implanted in various nuclei of the forebrain and midbrain to permit the recording of electrical activity. The purposes and results of these recordings have been reported elsewhere (23,26,27,85,86). At least two weeks were allowed for recovery after surgery. Each cat was then assigned to one of three groups for treatment with: (A) oral disulfiram (N = 10); (B) intraperitoneal amphetamine (N = 6); or (C) a combination of disulfiram and amphetamine (N = 10) .

The subjects in Group B received daily intraperitoneal injections of normal saline (1 ml) for 5 days before the administration of amphetamine was begun. Data collected on the fifth day were used as a control for changes induced by handling during injection Or by the injection itself. Treatment in this group consisted of two injections of methamphetamine hydrochloride daily, one at 9 AM and one at 3 PM. The dosage was increased from 15 mg/kg per day (7.5 mg/kg per injection) on the first day to 28 mg/kg on the eleventh day. No drug was administered on Days 6 and 7. All behavioral data for Group B animals were collected after the first injection on any given day.

The animals in Groups A and C were given disulfiram (in tablet form*) at 9 AM daily for 12 days. Daily dosage of disulfiram for each animal ranged from 150 to 180 mg/kg, depending on variations in its weight. In Group C, methamphetamine hydrochloride was also injected intraperitoneally each day at 3 PM, beginning on the second day of disulfiram treatment. In previous dosage trials, deaths caused by methamphetamine had been found to increase in frequency in animals pre-treated with disulfiram, particularly when the dosage range of

**The oral route of administration was used because repeated subcutaneous or intraperitoneal injections of disulfiram have been found to cause local irritative effects that distort quantitative behavioral data (68).*

amphetamine reached 15 to 30 mg/kg.* Lewander (58) has shown that there is no development of tolerance for the motor effects of amphetamine. Since our primary interest was to study such effects in Group C, the dosage of amphetamine in these animals was maintained well below the lethal level. Dosages of amphetamine used were 7.5 mg/kg on the first day and 10 mg/kg on subsequent days. On what would have been Days 6 and 7 of amphetamine administration, only disulfiram was given to cats in Group C, the amphetamine cycle being interrupted for two days as in Group B. The six-hour interval between the administration of disulfiram and that of amphetamine allowed time for the norepinephrine-depleting effects of disulfiram to develop.

Twenty minutes before the beginning of an experimental session, each animal was placed in a metal cage commercially available measuring 17.5 x 24 x 19.5 inches, to which a wooden floor and a Plexiglas door and walls had been attached. Data were gathered through direct observation of the cat's behavior and by 3-minute videotape recordings that were made at the following times: (a) on the two days prior to the administration of any drug; (b) six hours after the first dose of disulfiram; and (c) just before the daily injection of methamphetamine and 10,20,30 and 90 minutes after its administration. Thus, each subject served as its own control.

The animal's behavior was rated later after careful scrutiny of the videotape recordings, each 3-min recorded period being divided into 18 observational intervals, 10 seconds each. Scoring for each interval was made on a multidimensional rating chart, using a catalogue of elementary motor, postural, and autonomic phenomena that had been defined and coded as units of behavior in previous observations of a large number of cats before and after amphetamine treatment. A detailed presentation of the catalogue of definitions and of our extensive rating system has been made elsewhere (87). (For the reader's benefit, the definitions of special terms to be used through this paper are given in the Appendix.) Briefly, 27 categories of behavior are listed in the rating chart, including position and orientation of the animal in the cage, attitude, arousal, orientation and quality of the animal's gaze, presence of any grooming, sniffing, autonomic reactions, ataxia, dystonia or disjunction, movement speed, position and movements of the various body segments (head, trunk, forelegs, hindlegs, and tail) as well as modality and relatedness of any head stereotypy to that of other regions of the body. Within each category, the rater chooses from the catalogue those units that are most representative of the animal's actual behavior during the 10-sec interval. The separate rating of postures and movements for each region of the animal's entire body allows a detailed description and accurate quantitation of the drug effects on motor activity.

Movements and alterations of motor function were scored as present if they occurred at any time during an observational interval; postural features and attitudes, on the other hand, were considered present only when maintained for the major portion of an interval or for a longer period than other features and attitudes occurring within the same interval. Thus, when a cat had maintained the same posture or the same attitude for most of a 10-sec observational interval, the appropriate rating was assigned.

The psychomotor profiles of the animals in Groups A, B and C, as constructed from the evaluation of their attitudes and motor activities, are reported below.

RESULTS

Effects Of Disulfiram Administration (Group A):

A slight but consistent reduction in total spontaneous activity, as compared with that of the controls, was observed as early as 6 hours after the first dose of disulfiram (Fig. 1, top). The major decrease of movements was localized at the head-neck region and

**Fuller and Snoddy (35) reported increased sensitivity to the effects of exercise and lethality (within 3 hours) in rats treated with disulfiram and placed in a rotary drum turning at 8 rev/min, a situation not more exhausting than amphetamine-induced hyperactivity. This phenomenon in rats may be analogous to the increased lethality observed in our initial trials with increasing doses of methamphetamine.*

involved orienting activity. As a correlate, the attitudinal scales (Fig. 1, bottom) demonstrate that the

FIGURE 1

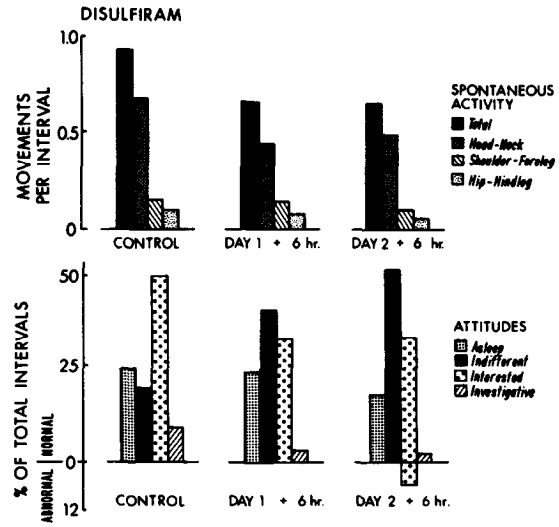


Fig. 1: Initial effects of disulfiram treatment on the amount of spontaneous activity and Occurrence of the observed attitudes. Control- before disulfiram treatment. Day 1+6 hr.- 6 hours after the first administration of disulfiram. Day 2+6 hr.- 6 hours after the second administration of disulfiram.

Interested and Investigative attitudes remained stable after the initial dose of disulfiram, but that the incidence of the Indifferent attitude was doubled on the first day, and was increased in frequency even further on the second day. On that day, however, spontaneous alerting responses in the form of startle reactions appeared*; an animal lying on the floor of the cage in an Indifferent attitude might suddenly, and for no apparent reason, raise its head and perform one or more fast orienting movements while adopting a tense Interested attitude for a short period. This startle activity is accounted for in the Abnormal Interested attitude shown in the graph. Similarly, it contributed to the scores for head-neck activity on the second day of treatment, which would otherwise have been expected to decrease because of the increase in Indifferent attitude.

With continued drug administration, all subjects developed a sleepy pattern of behavior that was manifest during the observational periods, or when the animal was left on the floor undisturbed. On these occasions, there was a marked preference for lying on one side, and an effort to regain the preferred side if tne animal's position was changed manually. By the end of the first week, somnolence and prolonged episodes of sleep from which the animals could be easily aroused, were predominant. Such episodes were, however, punctuated by not infrequent hyperactive startle reactions triggered by the occurrence of muscle twitches about the legs or body. Enhanced dopamine activity at the level of norepinephrine receptors subserving proprioceptive reflex mechanisms might explain the hyperactive quality of the response. both sleepiness and muscle twitches constituted early indications of neurotoxicity. In fact, hypotonia and weakness of the hind limbs were noted in most cats by the fourth day and were consistent with the ensuing manifestations (muscle wasting, ataxia, paresis) of an ascendant, irregularly progressive neuropathy. Impairment and subsequent loss of the righting reflex occurred between the fifth and tenth days. Recovery from all neurological disturbances occurred within 10 days after cessation of treatment, except in the case of one cat that died on the tenth day.

Comparative Effects of Methamphetamine Alone And Combined with Disulfiram (Groups B and C):*

Observations after a single dose of amphetamine: Despite some differences in degree, the immediate responses to amphetamine were similar in nature in

**The spontaneous occurrence of these reactions is not consistent with the sedative effect usually attributed to disulfiram and suggests thatits action is of a more complex nature than that of sedative agents, such as the barbiturates. In the same vein, Wise and Stein (91) described "disinterest in etectrical reinforcement" concurrent with the suppression of medial forebrain bundle self-stimulation in rats treated with acute doses of disulfiram, and differentiated this aspect of the animal's behavior from a sedative effect of the drug, since sedation after large doses of barbiturates, for example, does not necessarily interfere with self-stimulation and rats may respond at normal or supernormal rates.*

**A detailed account and phenomenological characterization of the behavioral effects of acute and chronic administration of amphetamine in the cat have been reported elsewhere (84). Therefore, reference will be made only to those aspects relevant to observations in animals pretreated with disulfiram.*

the animals in Groups B and C. A reduction in locomotor movements, coupled with increased muscular tension, arousal, and transient orienting responses of the head, occurred at the outset. In some animals, in either group, especially in those pretreated with disulfiram, postural fixation (i.e., maintenance of a single posture and the absence of any transient movement for a protracted period) with lack of orienting responses was a remarkable initial feature. Such inertia of the motor apparatus was revealed in responsive animals by particular signs of hypokinesia: maintenance of awkward postures due to lack or insufficiency of postural adjustments; drooling in the absence of altered deglutitory mechanisms (usually in the earlier periods of a recording session); suppression of automatic expressive movements of the face, e.g., reduced eye mobility, blinking activity, or changes in size of the palpebral fissures. The lack of mimicry was frequently associated with wide-open eyes and protracted visual fixation (staring response) resulting in a characteristic spaced-out physiognomy not unlike the "glazed expression" reported in cats treated with bulbocapnine and described as catatonic (21), augmented by a progressive bilateral mydriasis with diminished reaction to light. These manifestations of hypokinesia were, in general, more pronounced in animals pretreated with disulfiram. These same animals not infrequently displayed disparities in pupillary size through a full range of diameters, not only when the pupils were dilated. Although diminished reaction of the pupils to light was common to Groups B and C, unilateral failure to react was observed only in Group C (Fig. 2).

FIGURE 2

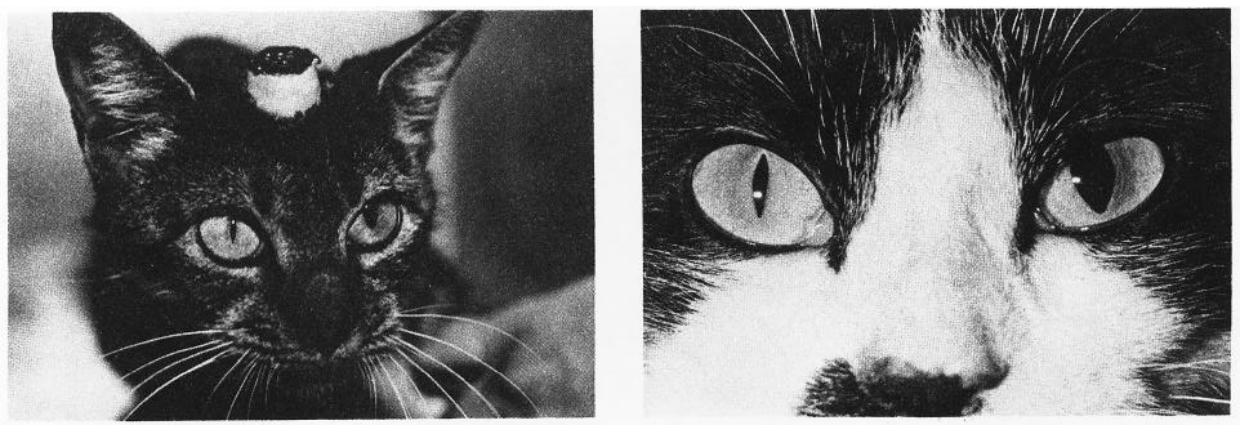


Fig. 2: Staring response and spaced-out facial expression following combined treatment. Unilateral autonomic dysfunction is illustrated by disparities in pupilltary size and expansion of the nictitating membrane.

The initial reduction in spontaneous activity provided a flat background against which the emergence of stereotyped behavior stood out sharply; such behavior occurred in all subjects within 15 minutes after the injection of amphetamine and consistently involved the head region. Fig. 3 shows the occurrence of total and regional stereotyped activity on the first day of amphetamine administration both in Group B and in Group C. The maximum level of stereotypy in the animals treated with amphetamine alone was reached at 30 minutes; eventually, during the last recording period, stereotyped activity involving the trunk (hunching or pivoting movements) supervened. Under combined treatment, peak stereotypy levels were reached earlier (at 20 minutes), and the early appearance of stereotyped movements in the trunk and leg regions (already present 20 minutes after the amphetamine injection) clearly contributed to the enhancement of the total stereotyped activity. Activation of dopamine systems in the relative absence of coordinating and inhibiting effects of norepinephrine systems, as a result of its depletion by disulfiram, could indeed be related to these observations. Interestingly, in the first 10-minute recording period, stereotypy did not occur as frequently as it did in the animals receiving amphetamine alone, a feature obviously related to more frequent initial postural fixation in pretreated cats.

FIGURE 3

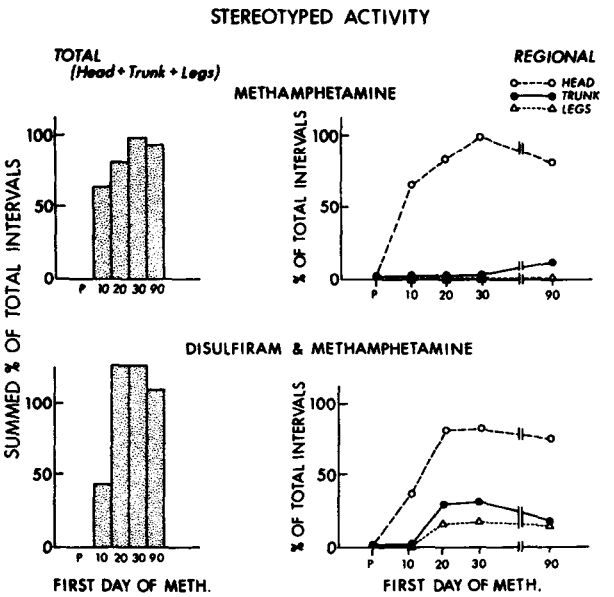


Fig. 3: Presence of stereotyped activity in each region of the body and the sum of the regional occurrences (total) on the first day of methaphetamine administration in Groups B and C. P=immediately preceding the first methamphetamine injection. 10, 20, 30, 90= 10, 20, 30, 90 minutes after the first methamphetamine injection.

Stereotyped head movements were performed in a relatively fixed sequence and developed into the motor components of exploratory patterns of looking and/or sniffing modalities.* On those occasions in which the pattern of stereotypy was interrupted by a pause or by transient head movements, it was noticed that these elements might be incorporated into the sequence of movements, changing the stereotypy into a new configuration or a combined modality, just as the imperceptible turn of a kaleidoscope causes a derived pattern to take shape. Later on, either the incorporated or the initial component of the pattern might be dropped out, the original modality being conserved or changed. Eventually, in animals treated with amphetamine alone, the head-neck stereotypy most frequently evolved into a constricted sniffing pattern ("minutiae search"), with a pecking-like oscillation of the head.

Stereotyped head movements, grouped according to their pattern modality, occurred under both experimental conditions, as illustrated in Figure 4 (left panel). In the animals receiving amphetamine alone, the looking modality was the predominant response in the earlier recording periods (10 and 20 minutes), but its incidence decreased markedly in the last one (90 minutes). In contrast, the sniffing modality showed a late increase of incidence, with highest levels at 90 minutes. Disulfiram pretreatment differentially affected the occurrence of both head-pattern modalities. Thus, while the looking modality was a sustained response in the last recording period, the sniffing modality showed a higher early incidence (at 20 minutes) and a marked decrease at 90 minutes. A differential participation of anatomical substrates, modulated by varying activity of neurotransmitters

**The qualification of a stereotypic pattern as of the looking or sniffing modality does not imply an afferent triggering stimulus; it merely suggests that the motor components of the pattern are similar to those observable in either of these behaviors.*

FIGURE 4

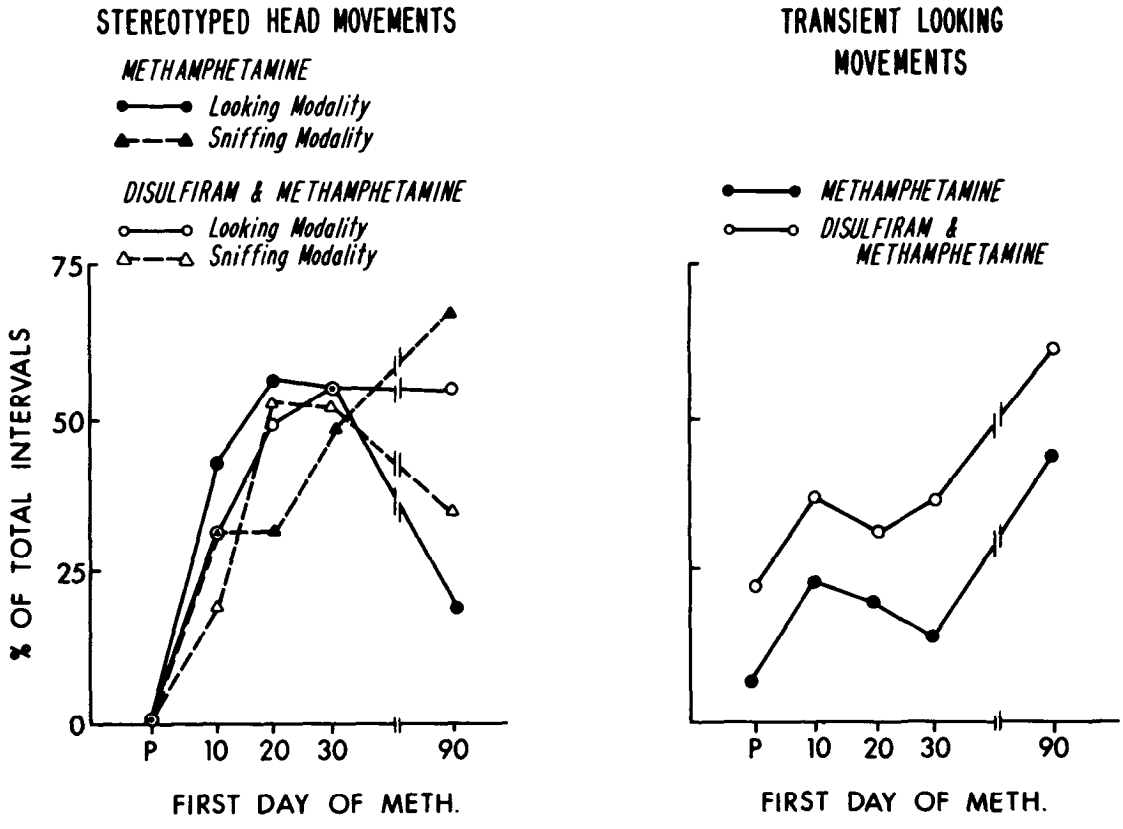


Fig. 4: Stereotyped and transient head movements in Groups B and C on the first day of methamphetamine administration. P=immediately preceding the first methamphetamine injection. 10, 20, 30, 90= 10, 20, 30, 90 minutes after the first methamphetamine injection. The summed incidence of both modalities does not reflect the total stereotyped activity of the head-neck region, since these activities may occur as a combined pattern of stereotypy (minutiae-side-to-side, for example).

involved in the elicitation of the stereotypy patterns, might be reflected in these changes.

The time course of the concurrent transient looking movements remained unmodified under combined treatment (Fig. 4, right panel), but the movements were more frequent in pretreated animals during all periods, including the pre-injection period. This increase appeared to be related to the occurrence of the previously described startle reactions induced by disulfiram. The sustained occurrence of transient looking movements observable at 30 minutes under combined treatment, as opposed to the slight decrease after treatment with amphetamine alone, was correlated with the development of a Reactive attitude in pretreated animals (see below). In both cases, a significant increase in frequency, as well as in speed and suddenness, of tne transient activity occurred at 90 minutes, when most Cats were highly aroused and hyperactive.

The selectivity of the amphetamine effect on muscle tone of the entire body was usually expressed by differences in local patterns of involvement; muscles acting on either side of a joint appeared to be unequally affected by increase in tone, resulting in the development of an awkward position for that body region. Such manifestations of regional dystonia were also more intense and appeared earlier in pretreated animals than in those receiving only amphetamine. Most intense and persistent was

a cataleptoid extensor leg posturing that we have called "abortive grooming" because it represents the initial component of a never completed grooming behavior. Thus, not infrequently, the haunch of a sitting cat would slide to a lying position (supported by a side on the floor) with both hind limbs simultaneously extended, one of them being raised and placed behind the animal's head and the other between the forelegs. On other occasions, in an animal leaning against a wall of the cage, a foreleg might also be involved in such extensor response (Fig. 5). The resulting contorted postures were usually preserved in spite of the concurrent transient or stereotyped activity of the head, and constituted a clear expression of dissociation between the animal's postural base and the superimposed movements and attitude, as observed on later days under treatment with amphetamine alone.

Similarly, the typical propped-up posture ("camel posture") usually seen in Group B at the height of intoxication in subsequent days occurred *in* most subjects in Group C on Day 1 during the periods of hyperkinesia and maximum arousal. This standing posture consists of a stiff appearance, wide-based hyperextended hind legs, arched back, and tail showing a waxy immobility (analogous to the Straub's tail-raising phenomenon in mice and rats given opiates or L-Dopa), and it was sustained even while the cats were peering about the environment and actively moving the head and extremities. The peculiar plastic rigidity of the tail (Fig. 6) was a long-lasting feature.

FIGURE 5

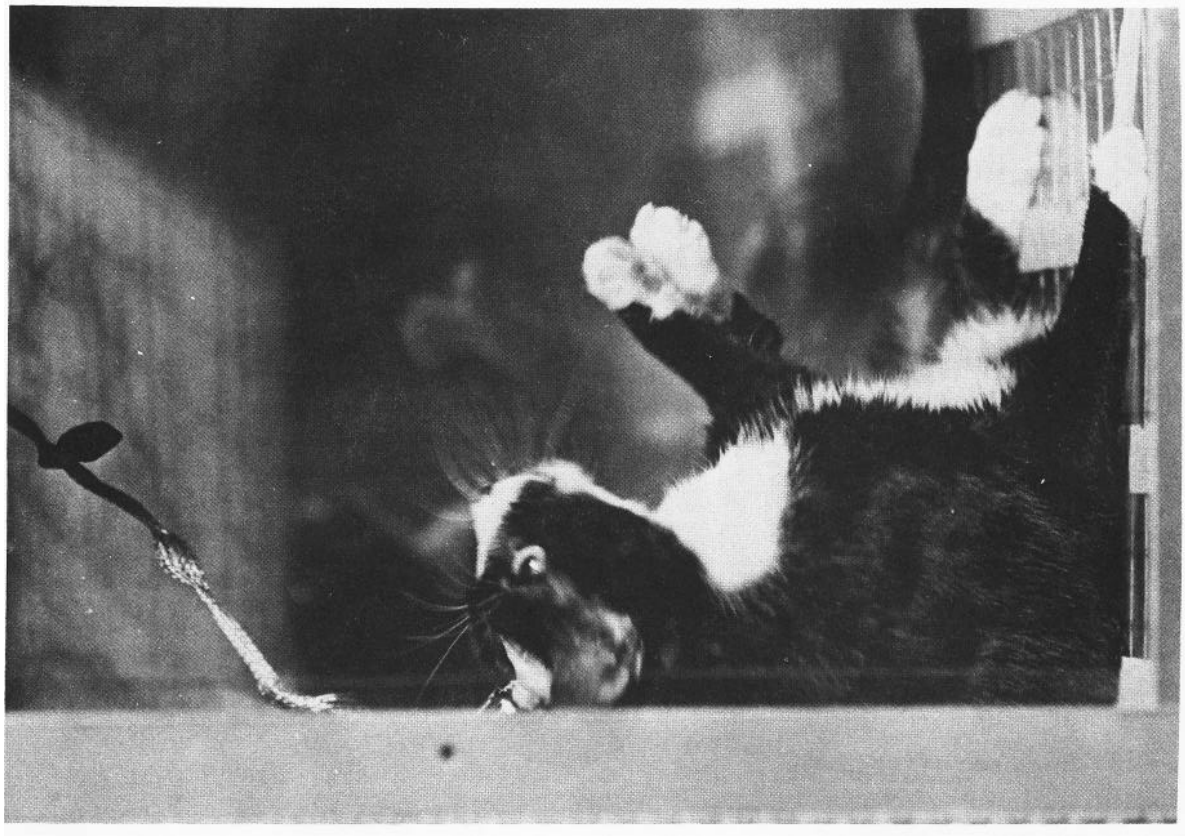


Fig. 5: Extensor leg posturing (abortive grooming) involving both hind- and fore-legs in a disulfiram pretreated animal.

FIGURE 6

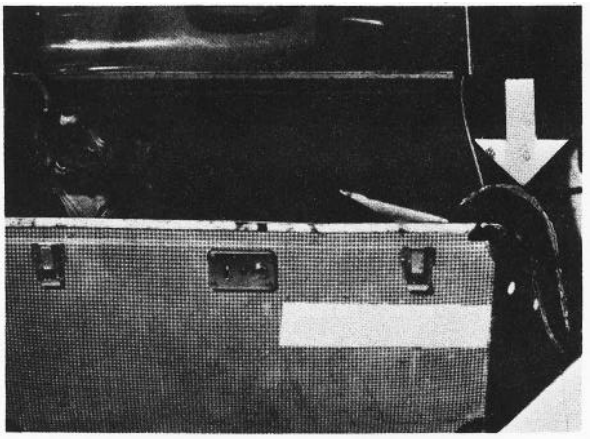


Fig. 6: Preservation of abnomal posture of the tail (waxy immobility), seen to last more than 20 minutes, 2 hours after the amphetamine injection in a cat pretreated with disutfiram.

Another manifestation of behavioral disjuntion that appeared earlier under combined treatment than with amphetamine alone was akathisia, which was expressed in both sitting and standing postures by restless leg repositioning or stepping and a vacillating maintenance of posture, as if a change of the animal's location within the cage might occur imminently. As illustrated in Fig. 7, akathisia was displayed only by pretreated animals on the last recording period of the first day. Dystonia, ataxia, and disjunction also reached a peak in these animals much earlier than in those treated only with amphetamine. Localized dyskinetic phenomena, such as myoclonic jerks, orofacial dyskinesias, or paw-shaking movements, occurred, on the other hand, later in the intoxication cycle under both experimental conditions.

FIGURE 7

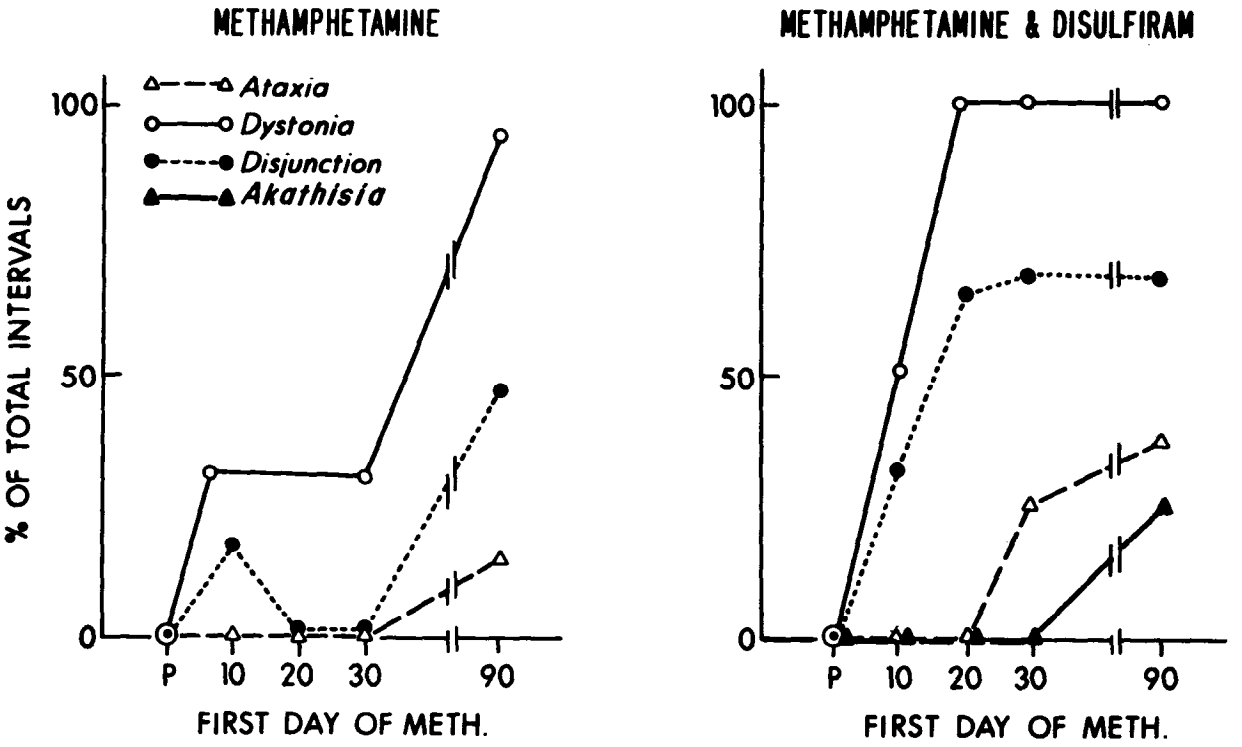


Fig. 7: Occurrence of ataxia, dystonia, disjunction and akathisia on the first day of methphetamine administration in Groups B and C. P= immediately preceding the first amphetamine injection. 10, 20, 30, 90= 10, 20, 30, 90 minutes after the first amphetamine injection.

FIGURE 8

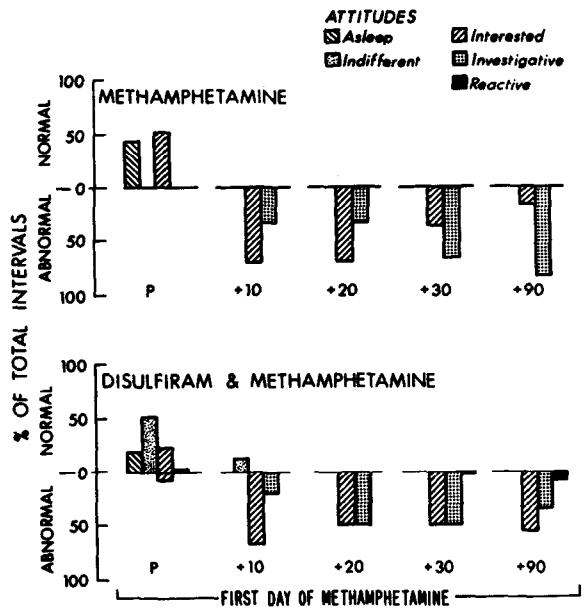


Fig. 8: Effect of disulfiram pretreatment (Group C) on the distribution of attitudes on the first day of methamphetamine administration. P= immediately preceding the first methamphetamine injection. 10, 20, 30, 90= 10, 20, 30, 90 minutes after the first methamphetamine injection.

The general psychomotor pattern of response, as represented in the attitudinal scales, is shown in Fig. 8. An abnormal quality of the animal's attitude was detectable shortly after the injection of amphetamine, with the attitude scores being similar for Groups B and C during the 10-minute recording period. In pretreated cats, however, the prevailing Indifferent attitude induced by disulfiram (pre-injection period) was still present 10 minutes after the administration of amphetamine and paralleled the already mentioned decreased occurrence of the sterotyped motor response. At variance with the observations on animals treated with amphetamine alone, in which the Investigative attitude became predominant during later recording periods, was the sustaining of the Interested attitude scores through all periods under combined treatment. More importantly, a Reactive attitude (usually the outcome of repeated injections over several days in cats receiving only amphetamine) was noted in pretreated animals at 30 and 90 minute after the first injection, manifested as exaggerated sensitivity toward the environment, with frequent attentional shifting, over-reactive movements and signs of hallucinatory behavior (visual tracing, aimless paw

TABLE 1

	GROUP A DISULFIRAM ALONE	GROUP B AMPHETAMINE ALONE		GROUP C DISULFIRAM AND AMPHETAMINE	
BEHAVIOR MEASURED		10-30 Min.	90-Min.	10-30 Min.	90-Min.
INTERESTED ATTITUDE	<.01	<.01	NS	<.01	<.01
INVESTIGATIVE ATTITUDE	NS	<.01	<.01	<.01	<.01
REACTIVE ATTITUDE	NS	NS	NS	NS	<.01
STEREOTYPY	NS	<.01	<.01	<.01	<.01
TRANSIENT MOVEMENTS	<.01 (decrease)	NS	NS	<.01 (increase)	<.01 (increase)
AKATHISIA	NS	NS	NS	NS	<.01
DISJUNCTION	NS	<.05	<.01	<.01	<.01
DYSTONIA	NS	<.01	<.01	<.05	<.01
ATAXIA	NS	NS	<.01	<.05	<.01

Statistical significance of recorded behavioral changes. All changes (except where indicated) represent an increase in the occurrence of the behavior in the treated animals from that in saline-treated controls. NS= no significant difference. Group A: 6 hours after the second dose of disulfiram; Group B: 10-30 minutes and 90 minutes after a single dose (7.5 mg/kg, ip) of methaphetamine; Group C: 10-30 minutes and 90 minutes after the first injection of methamphetamine in pretreated animals.

movements, or eluding jumps apparently related to non-existent objects in the environment).

A summary of the statistical significance of various behavioral changes observed on Day 1 under the three experimental conditions is presented in Table I. The figures are based on comparisons with control data collected on the fifth day of the initial period of treatment with saline and on the pre-drug periods.

Observations after repeated doses of amphetamine: Generally, a comparable evolving pattern of behavior was observed in animals in Groups B and C. Consistent trends connected with the acute effects of amphetamine were apparent for most behavioral responses in any recording session during the intoxication cycle. The cumulative effects of the drug differed from those in the naive animal, i.e., there was a shorter latency of the induced manifestations and an increase of transient activity, including adventitious movements bearing little or no relationship to the main vector of behavior.

However, the combined use of amphetamine and disulfiram resulted both in an accelerated development of the neuropathy caused by repeated administration of disulfiram alone, as described above for the animals in Group A, and the appearance, in several animals, of electrographic or generalized seizures. Interestingly, whereas manifestations of neurologic derangement were evident in the early days during the periods between amphetamine injection, or after its acute effects had worn off, the consequence of muscle weakness and progressive wasting could be detected during the recording sessions by the evaluation of certain motor activities that usually are increased under treatment with amphetamine alone (Fig. 9). Movements that require the active

FIGURE 9

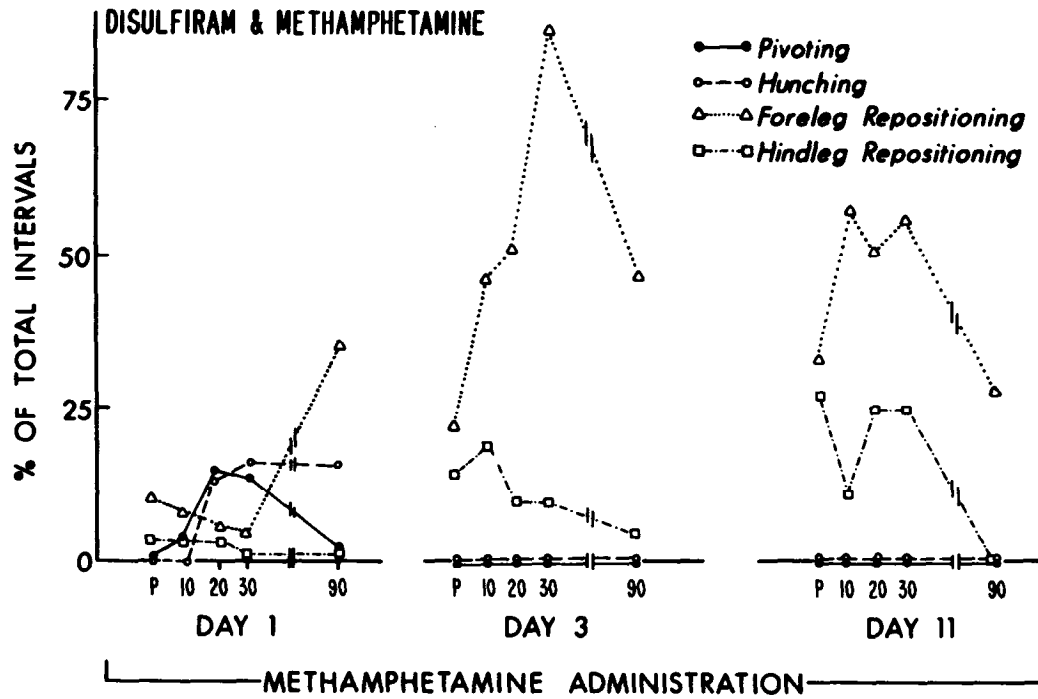


Fig. 9: Effects of developing neuropathy on specific motor units during continued treatment with disulfiram and methamphetamine (Group C). Day 1, 3, 11= first, third and eleventh days of methamphetamine administration. P= immediately preceding the first methamphetamine injection of the day. 10, 20, 30, 90= 10, 20, 30 90 minutes after the first methamphetamine injection of the day.

support of the hind limbs (hunching and pivoting), although present on Day 1, did not appear thereafter. On the contrary, hindleg and foreleg repositioning, movements that require much less support than do hunching and pivoting, were still performed on the eleventh day when the disulfiram-induced paresis had already become obvious. No comparison of the quantitative aspects of behavior in Groups B and C can legitimately be made for any day after the first. However, certain features induced by treatment with amphetamine alone were accentuated or affected qualitatively by pretreatment with disulfiram and deserve further comment from a phenomenological standpoint.

In contrast to the animals in Groups A and B, all subjects in Group C had impairment of the righting reflex about the third day of amphetamine injection (after four doses of disulfiram), and the reflex was completely lost within the first week. In many of these animals, as in those in Group A, unilateral postural preferences and resistance to attempts to change the laterality of the posture were noted during neurologic examination. Following the injection of amphetamine during the recording sessions, unilateral preference could be appreciated by the dominant direction (left or right for a particular cat) of stereotyped circling locomotion, if any.

Within the initial recording periods of each successive day, marked deficiencies of postural adjustments, as well as other signs indicative of alteration of the motor initiative, were particularly noticeable in Group C. For example, animals receiving only amphetamine might occasionally lean against the cage wall while executing a repetitive skating movement in which a foreleg slides outwards along the floor and is repositioned after considerable spread has occurred. Not only did this activity occur more frequently in pretreated animals, but it was obtunded, since the adjusting repositioning movement of the foreleg occurred only with maximum spread and after a protracted period (Fig. 10). When the animal was in locomotion, sudden and transient "freezing" of movement in mid-air could be observed; a cat might suddenly stop a circling locomotor movement with a hind leg already lifted for the next step remaining frozen in the air. On

FIGURE 10

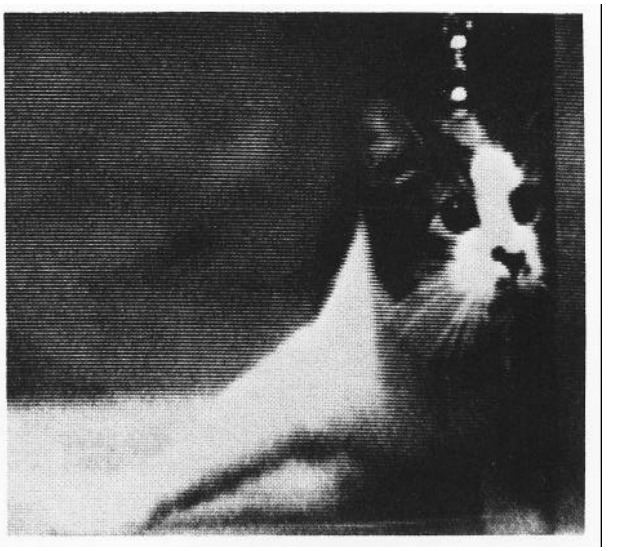


Fig. 10: Maintenance for a 15-minute period of abnormal posture in the absence of postural adjusting movement, observed in a pretreated animal (Day 3, 10-min period). Note spaced-out facial expression and drooling. Videotape picture.

other occasions, a foreleg would reach the ground crossed in front of the other one and the animal would freeze in that position for a while. Analogous phenomena recorded during experimental catatonia in cats and monkeys and in catatonic patients have been described as "barrage" of the psychomotor initiative (21). Manifestations of hypokinesia were also observed in the later periods of hyperkinesia, intermingled with reactive motor activity; a cat might retain uncomfortable postures that resulted from overreactive jumps apparently triggered by non-existent stimuli while still responding to the "imaginary" object (Fig. 11).

The tendency to adopt and retain a crouching posture in which the forelegs were spread out, a manifestation of regional dystonia only rarely observed in animal's given amphetamine alone, developed further under combined treatment. Toward the final days of the experiment, six of the ten pretreated cats demonstrated a locking of the proximal joints of the forelegs in such an abnormal range that the legs lay flat on the floor in a position almost perpendicular to the longtitudi-

FIGURE 11

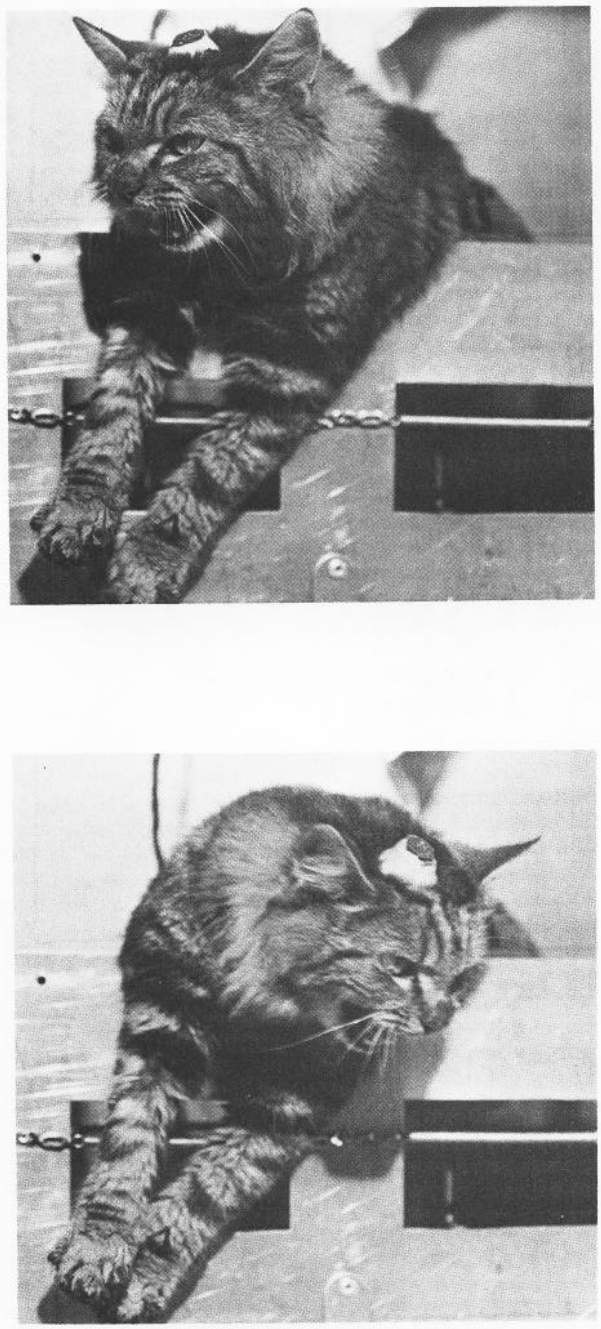


Fig. 11: Maintenance of abnormal posture concurrent with reactive movement toward "Imaginary" object during the hyperkinetic stage in an animal pretreated with disulfiram (Day 3, 65 minutes after the injection of amphetamine). Note facial expression and drooping of whiskers.

FIGURE 12

Fig. 12: Crucifixion posture following treatment with disulfiram and amphetamine (Day 11 75 minutes after the injection of amphetamine). Note piloerection and clawing.

nal axis of the body (Fig. 12). This bizarre posture persisted for at least two hours after the injection of amphetamine and resembled the "crucifixion posture" described in bufotenine or LSD-intoxicated monkeys (31) and in experimental and human catatonia (21).

Most dramatic, however, was the effect of chronic drug combination on the characteristic motor response to amphetamine, the stereotyped activity of the animal's head. An outstanding feature of this response during the heigt of amphetamine intoxication is the compulsion attached to the performance of repetitive movements, as shown by the cats' undistractibility and failure to respond to environmental stimuli that would normally attract their attention (e.g., noise, light, catnip, rat). Athough the stereotypy was prolonged, its increased intensity in pretreated animals was manifest through augmentation of its compulsivity, rather than by a higher frequency. Thus, a smoother flow and cohesion of the sequential movements within patterned stereotypies, with incorporation of otherwise interfering transient movements and subsequent development of combined constricted patterns, such as minutiae-search-side-to-side or up-and-down-minutiae-search, were frequently observed. In no other circumstance under amphetamine alone was the compulsivity as striking as was the sight, in the final days of the combined condition, of an animal in a crucifixion posture struggling to perform the stereotypy, while feebly dragging its nose over the floor in a side-to-side movement (Fig. 13).

The particularly high level of compulsivity of the last days could also be appropriated by the occurence, in several animals in Group C, of episodes of forced locomotor propulsion comparable to the obstinate progression syndrome reported by Bailey and Davis (12). Although none of these cats could, at this stage, correctly maintain its stance and all showed stagger and swaying of the body, they would thrust the head forward blindly, seemingly unable to turn or to back up after having walked into a corner of the cage. Strong propulsive movements of the legs would immediately follow, until exhaustion caused their transitory interruption as the hind limbs remained spread out on the floor. If

FIGURE 13

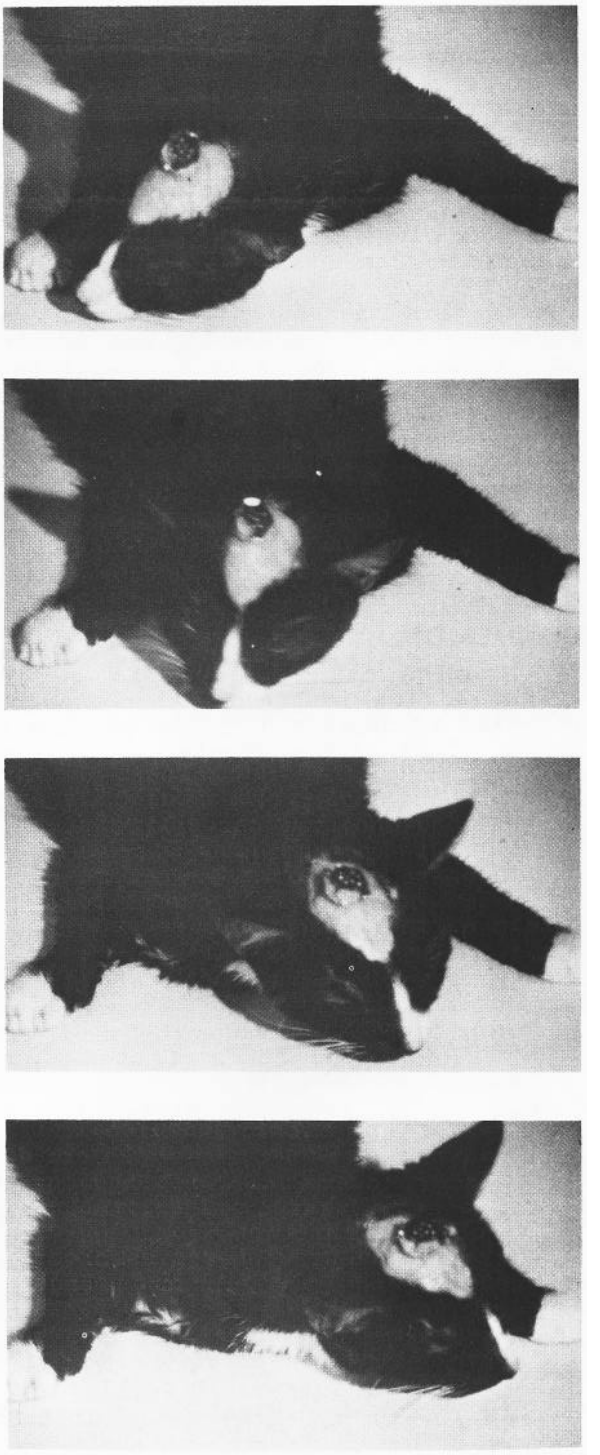


Fig. 13: Stereotyped side-to-side movement of the head concurrent with crucifixion posture in an animal pretreated with disulfiram (Day 11, 70 minutes after the injection of amphetamine).

the cat were placed in the center of the cage, it would keep its head forward and start pushing against the same corner or a different one. The appearance of obstinate progression was, at all times, concomitant with a highly aroused stupor-like state.

Finally, two animals in Group C demonstrated on the last day of the experiment a similar phenomenon to a "flashback": immediately after being placed in the observational cage and prior to the injection of amphetamine, these cats became hyperaroused and over-reactive. Apparently attempting to escape from some non-existent object, they made repeated hyperkinetic eluding jumps and locomotor movements until exhaustion made further activity impossible. The episode was accompanied by panting, tachycardia, unilateral mydriasis, urinary incontinence, and a facial expression connoting intense panic.

DISCUSSION

If dopamine is considered to be involved in the pathogenesis of schizophrenia, as suggested by the results of pharmacological, biochemical and clinical investigations referred to in the Introduction, then disulfiram meets several theoretical desiderata for an agent that can underline those effects of amphetamine intoxication germane to the endogenous psychosis.

1) There is evidence that disulfiram synergizes the potentiating effects of amphetamine on dopamine. Amphetamine is known to displace norepinephrine and dopamine from the neuronal stores and to inhibit their uptake into the nerve terminals (5,37). The inhibition of dopamine uptake, with consequent enhancement of dopamine activity, is greater at the level of the corpus striatum than in norepinephrine areas (19). In addition, the behavioral effects of amphetamine are closely related to the release of newly synthesized dopamine and norepinephrine (15). Disulfiram, on the other hand, via its inhibitory action on dopamine beta-hydroxylase, is effective in reducing the concentration of endogenous norepinephrine and increasing that of endogenous dopamine in the norepinephrine-containing regions of the brain (38,391. by decreasing the amount of norepinephrine relative to that of dopamine, disulfiram administration, in addition to potentiating dopamine activity in dopamine neurons, may cause the replacement of dopamine as a modulator of activity in norepinephrine neurons, since it is known that norepinephrine nerve terminals store and release dopamine when dopamine beta-hydroxylase is inhibited (4,46). These effects of disulfiram might be enhanced by using the drugs in combination. The blocking action of disulfiram on the synthesis of norepinephrine would reduce the availability of this neurotransmitter and, conversely, the releasing action of amphetamine would be expected to hasten the depletion of norepinephrine after disulfiram administration.

2) It is known that the absorption of disulfiram after its administration in vivo is followed at once by chemical reactions leading to complete reduction of the disulfide (38,83). Both disulfiram (28,61) and its main metabolites, diethyldithiocarbamate (65,74) and carbon disulfide (60), have been shown to increase the intensity, the duration, or both, of amphetamine-induced stereotypy (a proposed model psychosis per se): In addition the possibility exists that disulfiram may produce stereotypy, since this effect has been produced by FLA-63, an inhibitor of dopamine beta-hydroxylase which is structurally related to disulfiram (3).

3) Disulfiram may induce schizophrenic-like psychotic episodes. Such episodes on occasion clinically indistinguishable from schizophrenia (11), have been reported as a secondary development of the use of disulfiram in the treatment of chronic alcoholic patients (40,59,63,90). Although the risk of activating psychotic conditions is acknowledged by the contraindication of the use of disulfiram in their presence, the ability of the drug to exacerbate schizophrenic tendencies, especially of the paranoid type, generally contraindicates its use even if behavioral adjustments are only "borderline" (40).

4) Disulfiram has been shown to exaggerate profoundly the typical symptomatology in schizophrenic patients (43).

Additionally, because of its inhibiting action on dopamine beta-hydroxylase, disulfiram may mimic state of dopamine beta-hydroxylase a deficit that has been implicated as a mechanism for

the involvement of either dopamine (56) or norepinephrine in schizophrenia, the latter on the basis of experiments with 6-hydroxydopamine (81) and of findings in postmortem brain specimens schizophrenic patients (92). Further indirect support for these implications iS provided by the enhancement of psychotic manifestations in manic patients treated with fusaric acid, a blocker of dopamine beta-hydroxylase that possesses a relatively high degree of specificity (77).

Before the inhibiting action of disulfiram on dopamine beta-hydroxylase had been demonstrated, Heath et al. (43) studied the effects of the drug, given at an average dose of 1 g/day during 14 to 17 days, in nine chronic schizophrenic patients and nine prisoner-volunteers lacking personal or familial history of nervous or mental disease. Whereas all the subjects displayed, at the time of maximum effect (14 days), symptoms of neurotoxicity such as lethargy, reduced stream of thought, abbreviated memory span, and impaired ability to calculate, no alteration of thought processes and only increased emotional ability were recorded in the control group*. All the schizophrenic patients, on the other hand, experienced severe withdrawal and exaggeration of all schizophrenic symptoms. Thus, although secondary symptoms were particularly affected (auditory hallucinations, for example, developed de novo in eight patients and were accentuated in the remaining one), the primary ones were also heightened. Autism, inappropriate affective responses to the environment, and disturbance of thought processes gradually became more prominent, the latter becoming bizarre and with marked defects of association that precluded communication with the psychiatrists and ward personnel; total loss of previously intact orientation occurred in four of these patients, and akathisia was manifest in three others. In addition, schizophrenic patients complained of tightness of the legs after medication**.

**One exception was a subject with temporal lobe abnormality, as demonstrated etectroencephalographically before medication, who developed fill-blown psychotic symptoms toward the end of the treatment period.*

***Such complaint was also recorded in the VOlunteer with temporal lobe dysfunction.*

Whereas disulfiram is a non-specific inhibitor of dopamine beta-hydroxylase and causes multiple metabolic changes, the lack of development of schizophrenic-like or psychotic symptoms in the control subjects, as well as the accentuation of primary and secondary symptoms in schizophrenics, particularly the occurrence of thought disorders in contrast to their absence in the control group, clearly suggest that whatever alteration in the metabolism of catecholamines was provided by disulfiram, it was highly significant regarding the basic mechanisms of the disease. (Stronger support for a link between deficient activity of dopamine beta-hydroxylase and exacerbation of psychosis has recently been provided by the study with fusaric acid mentioned above.)

Consideration of this background suggested the current study to determine whether given end-stage components of the amphetamine-induced behavioral syndrome could be influenced by blocking the synthesis of norepinephrine. Table II presents our triple-layered model, with amphetamine psychosis serving as a model of functional paranoid psychosis, and changes of behavior induced by chronic amphetamine intoxication in the cat serving as a model of amphetamine psychosis. In the early days of intoxication, the restrictedness and directedness of the animals' behavior is reflected in their "postural-attitudinal" set, as well as in the stereotypic responses. As intoxication progresses, however, there is an increasing dissociation or "disjunction" between the animal's underlying postural base and the superimposed movements and attitudes. Disjunction, as we have defined it in our observations, represents a given body segment taking on an autonomous movement or position which does not seem to have a purpose or any relatedness to other body segments, e.g., repetitious repositioning of the forelegs occurring while a sniffing or looking pattern of stereotypy of the head develops, but lacking any apparent relationship to this pattern. Thus, the performance of movements unrelated to the main vector of behavior or the maintenance of a posture inappropriate for the activity being displayed characterizes the bizarre, disjunctive behavior-posture relationship of this stage of the intoxication cycle. Not

TABLE 2

FUNCTIONAL PARANOID PSYCHOSIS	HUMAN MODEL PSYCHOSIS Chronic Amphetamine Intoxication	ANIMAL MODEL PSYCHOSIS Chronic Amphetamine Intoxication
Paranoid Tendencies	Curiosity → Repetitious Examining; Searching; Sorting Behavior	Abnormal Investigatory Attitude with Repetitious Activity
	Sustained Pleasurable Suspiciousness → Looking for Meanings; Minutiae	Restricted Repetitious Activity
Paranoid Psychosis	Ideas of Reference → Persecutory Delusions and Hallucinations → Fearful; Panic Stricken; Agitated; Over-reactive	Reactive Attitude

Triple-layered model of chronic amphetamine-induced bekavior as a model of functional paranoid psychosis.

infrequently, akathisia-like repositioning and stepping movements that have a starting-and-stopping quality similar to that noted in chronic schizophrenia or as part of the tardive dyskinesia syndrome are manifest in the later stages of intoxication. The final state is best illustrated by the animal's Reactive attitude, in which hypersensitivity to minor or inapparent stimuli in the environment results in over-reactive orienting responses or eluding jumps that, in turn, may trigger increasingly hyperkinetic reactions. At this point, the composite picture is one of an agitated state of increased muscular tension in which the breakdown of behavior is accompanied by marked hyperarousal and, in many animals, signs of hallucination.

In the current experiments, a faster development of certain end-stage components of the amphetamine behavioral syndrome was obtained by pretreating amphetamine-intoxicated animals with disulfiram. Thus, on the first day of combined treatment, an earlier and more prominent behavioral disjunction was recorded than had been obtained with amphetamine alone. The behavioral disjunction represents an increased compartmentalization or autonomy of body regions in regard to both posture and movement, and was manifest in a greater incidence and intensity of extensor leg posturing, frequent display and maintenance of a camel posture, occurrence of akathisia, and early and relatively pronounced occurrence of trunk and legs stereotyped activity on Day 1. On that day, toward the end of the recording session, several pretreated animals developed a Reactive attitude, one of the more prominent end-stage components of the syndrome in cats receiving repeated doses of amphetamine. In contrast with the facilitation of these behaviors, was the absence of dyskinesias and hyperreflexia on Day 1. It is possible that development of receptor supersensitivity is necessary for the appearance of localized dyskinetic phenomena and exaggerated reflex activity.

The early loss of the righting reflex in the animals receiving combined treatment would be in keeping with an accentuated depletion of norepinephrine in and around the vestibular nuclei, following release of norepinephrine by amphetamine (24) and blocking of its synthesis by disulfiram, or with a functional alteration at the level of the basal ganglia which are known to mediate gravity-oriented labyrinthine reflexes (57). Manifestations of unilateral preference, on the other hand, might be due to accentuation of an intrinsic neurochernical imbalance of the nigrostriatal system (51), with greater release from one side than from its contralateral counterpart.

Several other features that were either more evident or delayed under combined treatment can be related to manifestations in animals treated with bulbocapnine or different psychotogenic compounds. Thus, loss of motor initiative, as represented by lack of mimicry, a "barrage" phenomenon, and decreased adjusting postural movements have been reported in experimental catatonia induced by bulbocapnine. The camel posture in our animals is reminiscent of the one transitorily adopted by cats in a stressful situation and is not unlike the "kangaroo posture" described by Adey (1) in cats treated with LSD. It is also suggestive of the "crooked back" posture of cats receiving various mescaline derivatives (21). Underlying interactions with neurotransmitters such as serotonin, which is known to be released by administration of amphetamine (36), might explain these commonalities.

As a correlate, Tatetsu (88) has described amphetamine addicts who manifested catatonic and paranoid syndromes in which the facial expression appeared slightly stiff and apathetic and the motor activity was dull and retarded, with absence of associated expressive movements of the hands in talking or arm movements while walking. Tatetsu described as a characteristic feature of amphetamine intoxication in these patients when they were seated a peculiar rigidity of the trunk in extended position accompanied by head, arms, and leg movements; this same feature was frequently part of the prodromal phase of the catatonic state (most frequently a mixture of stupor and hyperkinesia) that appeared under heavy intoxication. Another catatonic-like phenomenon in amphetamine addicts, similar to the postural fixation that was prominent in the animals given the combined treatment, is the "overamping", state, a state of hyperaroused immobility that may be elicited by a single large intravenous dose of amphetamine (55).

Because of the occasional occurrence of catatonic symptoms or catatonic forms of amphetamine psychosis and the findings of disjunctive behavior, loss of motor initiative, loss of righting reflex, and pupillary changes, there is a need to resolve the "paranoid model" in any amphetamine model of psychosis to fit those bizarre motor behaviors. In a Previous publication we suggested, on the basis of results from the experiments described herein and of clinical data from the literature that manifestations not consonant with the phenomenology of the paranoid psychosis reflect a "catatonic" phase of the amphetamine intoxication process (25) which is reminiscent of human catatonic behavior. Certainly, many points of commonality exist between catatonic and paranoid schizophrenia, especially since both conditions may appear in different phases in the course of an individual's illness (54). In examining the catatonic manifestations and their parallels with the paranoid manifestations, stereotypy may be a common mode in both forms of schizophrenia.

Bleuler (14) listed a variety of motor disorders noted in catatonic patients that also appeared in other forms of schizophrenia. Under stereotypies, Bleuler included posture, attitude, thinking, and movement. He also mentioned stereotypies of drawing, writing, music, and speech (verbigeration), and described stereotypies of posture showing ma persistence. For example, catatonics may stare at the same spot for weeks at a time. In a manner similar to that of our chronic amphetamine cats, stereotypies of position are expressed in two ways, viz. the patients always select the same corner of the room and always go to the same place to carry on a particular activity. Other signs of motor disorder mentioned by Bleuler are muscular tension or rigidity, loss of spontaneous movement or initiative, negativistic attitude, automatic obedience, hyperkinesia, relative autonomy of single muscular groups, general lack of coordination of body segments (for example, arm and leg movements that do not relate in a synchronous fashion during walking, or having feet step irregularly in time and space), and stupor. A unilateral increase in tendon reflexes has been noted. Differences in size of the two pupils and the absence of pupillary reactions to both light and accommodation are frequent. Also, oculomotor reaction (nystagmus) to vestibular stimulation is reduced in schizophrenics. This inhibition is seen primarily in catatonic patients and is related either to caloric or galvanic stimulation (17,32). In addition, postural reactions to vestibular stimulation found to be inhibited in schizophrenia and the finding of significantly less swaying induced by rotation in schizophrenics suggest reduced activity of the ventibulo-spinal mechanisms (33,69). Ornitz (71) postulated that pathological vestibular mechanisms would be manifested in an inability to initiate and carry out purposeful actions that are ordinarily automatically regulated. This inability would lead, in adult schizophrenics, to the psychomotor slowing and catatonic episodes that have been so eloquently described and related to vestibular and proprioceptive disturbance by McGhie and Chapman (66) and Chapman (16).

Loss or inability of initiative might well be a common denominator that cuts across many of the symptoms, including frozen postures and stereotypies. An amphetamine-induced distortion of the mechanisms that regulate the continuous flow of behavior might be the factor underlying not only stereotypy but also the preservation of perceptual-attitudinal sets characteristic of chronic amphetamine intoxication in both man and animals (29).

In viewing the amphetamine-induced syndrome in this way, the induced constraint or restriction of attitude or perception would have the same under lying mechanism as exists in a postural-motor stereotypy. Similarly, thinking itself could become repetitious, secondary to perseveration in one of the same systems subserving perception and cognition. Thus a beginning or incipient paranoid schizophrenic or an individual in the beginning phase of the amphetamine psychosis might start with an all-pervasive attitude of curiosity, suspiciousness or both and the content of this attitude would become increasingly constricted. Under the influence of increasing arousal, agitation, or both, the individual's behavior might then evolve into a paranoid state. Alternately, taking off from the same branching point of either stereotypy or restricted attitude, the catatonic pathway may

have a predilection to move in a motor or postural form because of a more intensive arousal or a more marked regression to lower levels of organization.

Although the current study may be criticized because of the neurotoxic effects of repeated administration of disulfiram, the fact remains that the over-reactive end-stage behaviors of the latter days of chronic amphetamine intoxication did occur under combined treatment, as represented by Reactive attitude on Day 1 (a time at which no sign of neurotoxicity was apparent in either Groups A or C), and that certain behaviors induced in the animals in Group B (amphetamine alone) were, at least qualitatively, enhanced by pretreatment with disulfiram in a direction consistent with comparable features in experimental catatonia and the catatonic form of schizophrenia. The more rapid development of end-stage behaviors in amphetamine-intoxicated animals pretreated with disulfiram can be explained as resulting from a stimulating effect of the drug combination in the relative absence of norepinephrine similar to that seen in chronic amphetamine intoxication, where there is also an imbalance in the ratio of norepinephrine to dopamine (42).

There is an interesting parallel between the accelerated development of peripheral neuropathy and the early occurrence of akathisia in animals pretreated with disulfiram, *on* the one hand, and the occurrence of tightness of the legs (a possible early manifesta- tion of peripheral neuropathy) and akathisia found in schizophrenic patients studied by Heath and asso- ciates, on the other. In addition to a possible greater vulnerability of these patients to the neurotoxic effects of the drug*, a correlation between the animal and the human conditions is suggested by the absence of the aforementioned symptoms in the control subjects.

The possibility that a correlation between induced behavioral manifesta- tions in animals and a psychopatho- logical condition in humans could provide new insights into the nature of mental derangements is an important one, and had been foreshadowed by T. S. Eliot, who wrote:

> *That Cats are much like you and me*
> *And other people* whom we find
> *Possessed* of various types of mind
> *For some are sane and* some are *mad.*

(from "The Ad-dressing of small Cats"
in *Old Possum's Book of Practical Cats*
by T.S. Eliot
by permission of Harcourt Brace Jovanovich, Inc.)

**One might speculate that this increased vulnerability may be related to abnormalities of musculature in schizophrenic patients which were first reported many years ago. Kraepelin (54), for example, refers to the not infrequent idiopathic muscular swellings concomitant with heiahtened sensibility of the muscles to percussion, and cites-Ajello's interpretation of peculiar muscle reactions to electrical stimuli in prospective catatonic patients as due to heightened irritability of the sarcoplasm. More recently, Meltzer (67) has postulated that changes in skeletal muscles occurring in psychotic patients are of mixed myopathic and neuropathic origin.*

APPENDIX

The assessment of behavioral alterations by means of direct observational technique requires concrete and specific definition of the units to be recorded. A limited meaning should, therefore, be assigned to terms that might, outside of this context, have various common usages. Following are the definitions not given in the text for such terms, as used in the current presentation.

Ataxia: Inadequate control of the range and precision of movements with or without quivering of flexed and/or extended regions of the body. It is as well applied to postural manifestations. Thus, while ataxia might be represented by marked motor inooordination, so that the animal tends to fall or is grossly unable to coordinate all four legs in order to walk or to maintain a standing posture, staggering or swaying movements in an animal keeping a certain posture are also indicative of its occurrence.

Attitude: Attentional mode and involvement of the animal, as manifested by its postural appearance and motor activity in relation to the surroundings and to its own body (environment). Whereas posture represents the endpoint of a movement or action, attitude reflects the quality of the animal-environment relationship. Classifications are Asleep: self-explanatory; Indifferent: lack of manifest attentional involvement with the environment; Interested: directed attentional contact with the environment or a part relationship. Classifications are Asleep: self-explanatory; Indifferent: lack of manifest attentional involvement with the environment; Interested: directed attentional contact with the environment or a part of it, but without definite physical approach; Investigative: selected attentional contact and active reaching out or approaching and examining of one or more parts of the environment. In the Interested attitude, the animal may single out aspects of the environment, but in the Investigative attitude an active interaction with those aspects is achieved through a selected approach response. Whenever these attitudes have a nervous, tense, or apprehensive quality, they are termed Interested Abnormal and Investigative Abnormal, respectively. The latter represents the persistent "compulsive" searching out of restricted "stimuli", often occurring even when the animal may have broken from a patterned (investigatory) motor stereotypy. Reactive: an abnormal attitude characterized by shifting attentional contact manifested in increased peering about the environment, frequent postural readjustments, and sudden inadequate or apparently unmotivated movements having a tense, agitated, jumpy quality.

Disjunction: Lack of proper relationship among postures or movements in two or more body regions, resulting in a fragmentation of the static or kinetic configuration of the body.

Dystonia: Abnormal postures or movements characterized by hyperflexion or hyperextension of body regions, resulting in an awkward appearance.

Hunching: Transitory elevation of the back forming a momentary hump or exaggerating an existing one. It may appear as a result of active backing up of forelegs against fixed hypertonic hindlegs or as compensatory movements associated with the execution of a stereotypy of the head.

Pivoting: Oscillating movement of the body around a fixed point, usually the hindquarter. Most frequently, the cage floor, with or without shifting of the body weight and without modification of the animal's place and orientation.

Stereotypy: Motor activity consisting of sequential repetition of movements describing a path of displacements recognizable as a kinetic configuration. Frequently observed stereotypic configurations that have been mentioned above are: Minutiae-search, pecking-like movements of the head executed by rotation around the interauricular axis; Side-to-side, horizontal head-neck movements around the base of the neck or around the occipito-atlantoid articulation; Up-and-down, vertical head-neck movements (smooth or jerky) around the base of the neck or around the occipito-atlantoid articulation.

ACKNOWLEDGMENTS

This work was supported in part by NIMH Grants 5 TO1 MH 07073 and MH 18904 and by Veterans Administration Funds. I thank Dr. E.H. Ellinwood, Director of the Behavioral Neuropharmacology Section, Duke University Medical Center, for providing the materials and physical facilities for this study. My thanks are also due to Linda Nelson and Nancy Wagoner for their expert technical assistance, and to Dr. David Frost for editing this manuscript.

REFERENCES

1. Adey, W.R., F.R. Bell and J.B. Dennis: Effects of LSD-25, psilocybin and psilocin on temporal lobe EEG patterns and learned behavior in the cat, Neurology, 12: 591-602, 1962.

2. Adams, R.N.: Stein and Wise theory of schizophrenia: a possible mechanism for 6-hydroxydopamine formation in vivo. Behav. Biol., 7:861-866, 1972.

3. Ahlenius, S. and E. Jörgen: Effects of a dopamine (DA)-β-hydroxylase inhibitor on timing behavior; Psychopharmacologia, 24: 243-246, 1972.

4. Anden, N.E., C.V. Atack and T.H. Svensson: Release of dopamine from central noradrenaline and dopamine nerves induced by a dopamine β-hydroxylase inhibitor. J. Neuronal. Trans., 34:93, 1973.

5. Anden, N.E., A. Carlsson, J. Haggendal: Adrenergic mechanisms. Annu. Rev. Pharmacol., 9:119:134. 1969.

6. Angrist, B. and S. Gershon: The phenomenology of experimentally induced amphetamine psychosis-preliminary observations. Biol. Psychiat., 2:95-107, 1970.

79 Angrist, B. and S. Gershon: A pilot study of pathogenic mechanisms in amphetamine psychosis utilizing differential effects of d and l-amphetamine. Pharmakopsychiat., Neuro-Psychopharmakol. 4:64-75, 1971.

8. Angrist, B., H.K. Lee and S. Gershon: The antagonism of amphetamine-induced symptomatology by a neuroleptic. Am. J. Psychiat., 131: 817-819, 1974.

9. Angrist, B., G. Sathananthan, s. Wilk and S. Gershon: Behavioral and biochemical effects of L-Dopa in psychiatric patients. Biochem. Pharmacol., suppl. (part 2), pp. 822-825, 1974.

10. Angrist, B., B. Shopsin and S. Gershon: Comparative psychotomimetic effects of stereoisomers of amphetamine. Nature, 234: 152-153, 1971.

11. Angst, Von J.: Zur Frage der Psychosen bei Behandlung mit Disulfiram (Antabus): Literatur über Sicht und Kasuistischer Beitrag. Schweiz. Med., 88: 1304-1306, 1956.

12. Bailey, P. and E.W. Davis: Effects of lesions of the periaqueductal gray matter in the cat. Proc. Soc. exp. Biol. Med., 51: 305-306, 1942.

139 Beamish, P. and L.G. Kiloh: Psychoses due to amphetamine consumption. J. Meni. Sci., 106: 337-343, 1960.

14. Bleuler, E.: Textbook of Psychiatry, New York, MacMillian, 1924.

15. Carlsson, A.: phetamine and brain catecholamines. in: Amphetamine and Related Compounds, E. Costa and S. Garattini (Eds.), Raven Press, New York, 1970, pp. 289-300.

16. Chapman, J.: Visual imagery and motor phenomena in acute schizophrenia. Brit. J. Psychiat., 113: 711-778, 1967.

17. Claude, H., H. Baruk and M. Aubry: Contribution a l'étude de la demence precoce catatonique: Inexcitabilité labyrynthique au cours de la catatonfe. Rev. Neurol., 1: 976-980, 1927.

18. Connell, P.H.: Amphetamine Psychosis. Maudsley Monograph No. 5, Oxford University Press, London, 1958.

19. Coyle, J.T. and S.H. Snyder: Antiparkinsonian drugs: inhibition of dopamine uptake in the corpus striatum as a possible mechanism of action. Science, 166: 899-901, 1969.

20. Davis, J.M. and D.D. Janowsky: Amphetamine and methylphenidate psychosis. Biochem. Pharmacol., suppl. (part 2), pp. 811-815, 1974.

21. DeJong, H.: Experimental Catatonia, Williams and Wilkins Co., Baltimore, 1945.

22. Domino, E.F.: Is the indole hallucinogen model worthwhile pursuing? In: Predictability in Psychopharmacol-onv-Clinical and Preclinical Correl-ations, A. Sudilovsky, S. Gershon and B. Beer (Eds.), Raven Press, New York 1975, pp. 247-268.

23. Ellinwood E.H.: Behavioral and EEG Changes in the Amphetamine Model of Psychosis. In: Neuropsychopharmacologv of Monoamines and Their Regulatorv Enzymes, E. Usdin (Ed.),Raven Press, New York, 1974, pp. 281-197.

24. Ellinwod, E.H. and O. Escalante. Behavioral and histopathological findings during chronic amphetamine intoxication. Biol. Psychiat., 2: 27-39, 1974.

25. Ellinwood, E.H. and A. Sudilovsky: Chronic amphetamine intoxication: behavioral model of psychoses. In: Psychopathology and Psyhopharmacology J.O. Cole, A.M. Freedman and A.J. Friedhoff, (Eds.), The Johns Hopkins University Press, Baltimore, 1973, pp. 51-70.

26. Ellinwood, E.H. and A. Sudilovsky: Arousal and EEG changes in the amphetamine model of psychosis. J. Pharmacologic, 5: 27, 1974.

27. Ellinwood, E.H., A. Sudilovsky and R. Gabrowy: Olfactory forebrain seizures induced by methamphetamine and disulfiram. Biol. Psychiat., 7: 89-99, 1973.

28. Ellinwood, E.H., A. Sudilovsky and L. Nelson: Behavioral analysis of chronic amphetamine intoxication. Biol. Psychiat., 4: 215-230, 1972.

29. Ellinwood, E.H., A. Sudilovsky and L. Nelson: Evolving behavior in the clinical and experimental amphetamine (Model) psychosis. Am. J. Psychiat. 130:1088-1093, 1973.

30. Espelin, D.E. and A.K. Done: Amphetamine poisoning - effectiveness of chlorpromazine, N. Eng. J. Med., 278: 1361-1365, 1968.

31. Evarts, E.V.: Some effects of bufotenine and lysergic acid diethylamide on the monkey. A.M.A. Arch. Neurol Psychiat., 75: 49-53, 1956.

32. Fitzgerald, G. and E. Stengel: Vestibular reactivity to caloric stimulation in schizophrenics. J. Ment. Sci., 91: 93-100, 1945.

33. Freeman, H. and E.H. Rodnick: Effect of rotation on postural steadiness in normal and in schizophrenic subjects. Arch. Neurol Psychiat., 48: 47-53, 1942.

34. Friedhoff A.J. Biogenic Amines and Schizophrenia in Biological Psychiatry, J. Mendels [Ed), John Wiley & Sons, New York, 1973, pp. 113-129.

35. Fuller, R.W. and H. Snoddy: Inhibition by Disulfiram of the accelerated turnover of catecholamines in the adrenal glands of exercised rats. J. Pharm. Pharmacol., 20: 156-157, 1968.

36. Fuxe, K. and U. Ungerstedt: Histochemical, biochemical and functional studies on central monoamine

neurons after acute and chronic amphetamine administration. In: Amphetamine and Related Compounds, E. Costa and S. Garattini (Eds.), Raven Press, New York, 1970, pp. 257-288.

37. Glowinski, J. and J. Axelrod: Effect of drugs on the uptake, release and metabolism of H_3-norepinephrine in the rat brain. J. Pharmacol. Exp. Ther., 149: 43-49, 1965.

38. Goldstein, M.: Inhibition of norepinephrine biosynthesis at the dopamine-beta-hydroxylation stage. Pharmacol. Rev., 18: 77-82, 1966.

39. Goldstein, M. and K. Nakajima: The effect of disulfiram on catecholamine levels in the brain. J. Pharmacol. Exp. Ther., 157: 96-102, 1967.

40. Gottesfeld, H.B., M.L. Lasser, E.J. Conway and N.M. Mann: Psychiatric implications of the treatment of alcoholism with tetraethylthiuram disulfide, Quart. J. Stud. Alco., 12: 184-205, 1951.

41. Griffith, J.J., J. Oates and J. Cavanaugh: Paranoid episodes induced by drug J. Amer. Med. Assoc., 205: 39, 1968.

42. Gunne, E., T. Lewander: Long-term effects of some dependence-producing drugs on the brain monoamines. In: Molecular Basis of Some Aspects of Mental Activity. Wahaas, O. (Ed), Academic Press, New York, 1967, Vol 2, pp. 75-81.

43. Heath, R.G., W. Nesselhof, M.P. Bishop and L.W. Byers: Behavioral and metabolic changes associated with administration tetraethylthiuram disulfide (Antabuse). Dis. Nerv. Syst., 26: 99-105, 1965.

44. Hökfelt, T., K. Fuxe, 0. Johansson and A- Ljungdahl:
Pharmaco-histochemical evidence of the existence of dopamine nerve terminals in the limbic cortex. Eur. J. Pharmac, 25: 108-112, 1974.

45. Horn, A.S. and S.H. Snyder: Chlorpromazine and dopamine: conformational similarities that correlate with the antischizophrenic activity of phenothiazine drugs. Proc. Natl. Acad. Sci. USA, 68: 2325-2328, 1971.

46. Hornykiewicz. O.: Dopamine (3-hydroxytryptamine) and brain function. Pharmacol. Rev., 18: 925-964, 1966.

47. Hornykiewicz, 0.: Dopamine: its physiology, pharmacology and pathological neurochemistry. In: Biogenic Amines and Physiological Membranes in Drug Therapy, part B, J.H. Biel and L.G. Abood (Eds), M. Dekker Inc., New York, 1971, pp. 173-258.

48. Janowsky, D.S., M.K. El-Yousef, J.M. Davis and H.J. Sekerke: Cholinergic antagonism of methylphenidate induced stereotyped behavior. Psychopharmacologia, 27:295-302, 1972.

49. Janssen, A.J.: The evolution of the butyrophenones haloperidol and trifluperidol from meperidine-like-4-phenylpiperidines. Int. Rev. Neurobiol., 8: 221-263, 1965.

50. Janssen, P., C. Niemergeers, *K.* Schellekens and F. Lanaerts: Is it possible to predict the clinical effects of neuroleptic drugs (major tranquillizers) from animal data. Arzneim-Forsch., 17: 841-854, 1967.

51. Jerussi, T.P. and S.D. Glick: Apomorphine-induced rotation in normal rats and interaction with unilateral caudate lesions. Psychopharmacologia, 40: 329-334, 1975.

52. Kallant, O.J.: The amphetamines - Toxicity and Addiction. University of Toronto Press, Toronto, 1966.

53. Kety, S.S.: Toward hypotheses for biochemical component in the vulnerability to schizophrenia. Semin. Psychiat., 4: 233-238, 1972.

54. Kraepelin, E.: Dementia Praecox and paraphrenia. Translated by R.M. Barclay and G.M. Robertson: Robert Krieger Publishing Company, Huntington, N.Y., 1971.

55. Kramer, J.C., V.S. Fischman and D.C. Littlefield: Amphetamine abuse-pattern and effects of high doses taken intravenously. Amer. Med. Assoc., 201: 305-309, 1967.

56. Lamprecht, F., R.J. Wyatt, R. Belmaker, D.L. Murphy and W. Pollin: Plasma dopamine beta-hydroxylase in identical twins discordant for

schizophrenia. Biochem. Pharmacol., suppl. (part 2), 937-940, 1974.

57. Langworthy, O.R.: The Sensory Control of Posture and Movement. A review of the studies of Derek Denny-Brown. Williams and Wilkins Co., Baltimore, 1970.

58. Lewander. T.: On the accumulation of p-Hydroxynorephedrine in noradrenaline neurons during chronic administration of amphetamine in the rat in relation to amphetamine tolerance. Psychiatr. Neurol Neurochir., 75: 215-218, 1972.

59. Liddon, S. and R. Satran: Disulfiram (Antabuse) psychosis. Am. J. Psychiat., 123: 1284-1289, 1967.

60. Magos, L.: Amphetamine reduced stereotypy in carbon disulphide-exposed and diethyldithiocarbamate-treated male rats, In: The Correlation of Adverse Effects in Man with Observations in AnimUals, S.B. De C. Baker (Ed.), Exerpta Medica, Amsterdam, 1971, pp. 24-27.

61. Maj., J. and E. Przegalinski: Disulfiram and the effect of catecholamines on neuroleptic-induced catalepsy in mice and rats. J. Pharm. Pharmacol., 19: 68-76, 1967.

62. Mandell, A.J. and D.S. Segal: The psychobiology of dopamine and the ethylated indoleamines, with particular reference to psychiatry. In: Biological Psychiatry J. Mendels (Ed). John Wiley & Sons, New York, 1973, pp. 89-112.

63. Mann, N.M, E.J. Conway, H.B. Gottesfeld and M.L. Lasser, coordinated approach to antabuse therapy. J. Amer. Med. Assoc., 149:40-46, 1952.

64. Matthysse, S.: Antipsychotic drug actions: a clue to the neuropathology of schizophrenia? Fed. Proc., 32:200-205, 1973.

65. Mayer, 0. and V. Eybl: The effect of diethyldithiocarbamate on amphetamine induced behavior in rats. J. Pharm. Pharmacol., 23, 894-896, 1971.

66. McGhie, A. and J. Chapman: Disorders of attention and perception in early schizophrenia. Brit. J. Med. Psychol., 34: 103-116, 1961.

67. Meltzer, H.Y.: Central core fibers in an acutely psychotic patient. Evidence for a neurogenic basis for the muscle abnormalities in the acute psychoses. Arch. Gen. Psychiat., 27: 125-132, 1972.

68. Moore, K.E. and R.H. Rech: Behavioral and norepinephrine-depleting effects of disulfiram in reserpine-pretreated rats. Arch. Int. Pharmacodyn. Ther., 180: 413-422, 1969.

69. Myers, S., D. Caldwell and G. Purcell: Vestibular dysfunction in schizophrenia. Biol. Psychiat., 7: 255-261, 1973.

70. Norton, S.: The effects of psychoactive drugs on cat behavior. Ann. N.Y. Acad. Sci., 159: 915-927, 1969.

71. Ornitz, E. Vestibular dysfunction in schizophrenia and childhood autism. Compreh. Psychiat., 11: 159-173, 1970.

72. Randrup, A. and I. Munkvad: Biochemical, anatomical and psychological investigations of stereotyped behavior induced by amphetamines, in Amphetamines and Related Compounds, E. Costa and S. Garattini (Eds.), Raven Press, New York, 1970, pp. 695-713.

73. Randrup, A. and I. Munkvad: Pharmacology and physiology of stereotyped behavior. J. Psychiat. Res., 11: l-10, 1974.

74. Randrup, A. and J. Scheel-Kruger: Diethyldithiocarbamate and amphetamine stereotyped behaviour. J. Pharm. Pharmacol., 18: 752, 1966.

75. Rossum, J.M. van: The significance of dopamine-receptor blockade for the action of neuroleptic drugs, in Neuropsychopharmacology, H. Brill (Ed.) Excerpta Medica Foundation, The Hague,- 1967, pp. 321-329.

76. Rylander, G. and M. Holmberg: Stereotype behaviour in man following amphetamine abuse. Symposium on Addiction and Tolerance Amphetamines. A. Liljestrand (Ed), Uppsala, 1970, pp: 258-262.

77. Sack, R.L. and F.K. Goodwin: Inhibition of dopamine beta-hydroxylase

in manic patients, a clinical trial with fusaric acid. Arch. Gen. Psychiat., 31: 649-654, 1974.

78. Schirring, E.: Amphetamine induced selective stimulation of certain behavior items with concurrent inhibition of others in an open field test with rats. Behaviour, 39: l-17, 1971 l

79. Seeman, P. and T. Lee: Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science, 188: 1217-1219, 1975.

80. Snyder, S.H.: Amphetamine Psychosis: a "Model" schizophrenia mediated by catecholamines. Am. J. Psychiat., 130: 61-67, 1973.

81. Stein, L. and C.D. Wise: Possible etiology of schizophrenia: progressive damage to the noradrenergic reward system by 6- hydroxydopamine. Science, 171: 1032-1036, 1971.

82. Stevens, J.R.: An anatomy of schizophrenia? Arch. Gen. Psychiat., 29: 177-189, 1973.

83. Strömme, J.H.: Metabolism of disulfiram and diethyldithiocarbamate in rats with demonstration of an in vivo ethanol-induced inhibition of the glucuronic acid conjugation of the thiol, Biochem. Pharmacol. 14: 393-410, 1965.

84. Sudilovsky A.: Disruption of behavior in cats by chronic amphetamine intoxication, Int. J. Neurol. 10: 259-275, 1975.

85. Sudilovsky, A. and E.H. Ellinwood: Unpublished results in Discussion, Current Concepts of Amphetamine Abuse, E.H. Ellinwood and S. Cohen (Eds.). U.S. Government Printing Office, Washington, D.C., 1972.

86. Sudilovsky, A. and E.H. Ellinwood: EEG changes and motor impairment after disulfiram administration to cats. Fed. Proc., 32: 791, 1973.

87. Sudilovsky A., E.H. Ellinwood, F. Dorsey and L. Nelson. Evaluation of the Duke University behavioral rating inventory for drug-generated (BRIDGE). In: Predictability in Psychopharmacology - Preclinical and Clinical Correlatiotions, A. Sudilovsky, S. Gershon and B. Beer (Eds.), Raven Press, New York 1975, pp-189-212.

88. Tatetsu, S., A. Goto and T. Fujiwara: The Methamphetamine Psychosis. Igaku Shoin Ltd., Tokyo 1956, pp. 76 and 80.

89. Taylor, K.M. and S.H. Snyder: Differential effects of d- and l-amphetamine on behavior and on catecholamine disposition in dopamine- and norepinephrine-containing neurons of rat brain, Brain Research 28: 295-309, 1971.

90. Wallerstein, R.S.: Hospital Treatment of Alcoholism, Basic Books, New York, 1957.

91. Wise C.D. and L. Stein: Facilitation of brain self-stimulation by central administration norepinephrine. Science, 163: 209-211, 1969.

92. Wise, C.D. and L. Stein: Dopamine β-hydroxylase deficits in the brains of schizophrenic patients. Science, 181: 344-347, 1973.

AUTHOR

Abraham Sudilovsky, M.D.: E.R. Squibb and Sons, P.O. Box #4000, Princeton, New Jersey 08540.

THE EFFECTS OF HEROIN ON CATECHOLAMINE METABOLISM IN MAN

Joseph J. Schildkraut,
Roger E. Meyer,
Paul J. Orsulak,
Steven M. Mirin
Mark Roffman
Patricia A. Platz,
Edwin Grab,
Mary E. Randall
Mark McDougle

INTRODUCTION

Since the observation by Vogt (34) that morphine decreases the level of norepinephrine in cat hypothalamus and midbrain, numerous studies have examined the effects of morphine on catecholamines in the brain. In recent years many investigators have studied the effects of morphine on the turnover of catecholamines either in animal brain or in brain regions using several different methods including: the synthesis and accumulation of radioactively labeled norepinephrine and dopamine from radioactively labeled tyrosine; the rate of decline of intracerebrally administered radioactively labeled norepinephrine; the rate of decline of endogenous dopamine and norepinephrine after synthesis inhibition by alpha-methylparatyrosine; the accumulation of homovanillic acid (the principal metabolite of dopamine); and we have recently examined the effects of morphine on the accumulation of 3-methoxy-4-hydroxy-phenylglycol (MHPG) sulfate (the major metabolite of norepinephrine in rat brain (27).

Animal Studios

In a number of studies, acutely administered morphine has been found to increase the turnover of dopamine in whole rodent brain as well as in the striatum and other brain regions, as determined by the conversion of radiolabeled tyrosine to radiolabeled dopamine (4,6,11,15,17,31,32), the rate of depletion of dopamine after inhibition of its synthesis (13,22,33), and the accumulation of homovanillic acid (1,10,16). After chronic administration, tolerance to the effects of morphine on the turnover of dopamine in brain has been observed in several studies (6,13,26,32), but not in all (4,15). During withdrawal from chronic morphine, the turnover of dopamine in brain appears to be decreased (13,26).

In several studies, after acute administration of morphine, an increase in norepinephrine turnover has been observed both in whole mouse brain and

in mouse cortex, cerebellum, brain stem and diencephalon as well as in rat hypothalamus as determined by the synthesis of "C-norepinephrine from ^{4}C-tyrosine (15,31,32), and in rat pons-medulla but not hypothalamus as determined by the rate of depletion of norepinephrine after synthesis inhibition (33). However, in several other studies, acutely administered morphine did not appear to alter the turnover of norepinephrine in brain as determined by the synthesis of ^{4}C-norepinephrine from ^{4}C-tyrosine (4,5,6,) or the rate of depletion of norepinephrine after synthesis inhibition (13).

In our studies, the acute administration of morphine produced an increase in norepinephrine turnover in whole rat brain as determined by the levels of MHPG-SO, in brain (24,25). After chronic treatment with morphine, tolerance developed to this morphine-induced increase in the levels of MHPG-SO_4 in rat brain (24,25). These findings support the observations of another group of investigators (26,32) who used other techniques to demonstrate tolerance to the morphine-induced increase in norepinephrine turnover in mouse brain. However, a persistent increase in norepinephrine turnover in either whole rat brain or rat brain regions has been observed after chronic administration of morphine in other studies (4,15).

After spontaneous withdrawal from chronic morphine, Rosenman and Smith (26) observed a decrease in norepinephrine turnover in mouse brain with return to normal by 66 hours after withdrawal, while Neal (21) reported no change in norepinephrine turnover in rat brain 60 hours after morphine withdrawal. In our studies, the level of MHPG-SO_4 in brain was significantly reduced 16 hours after withdrawal of morphine from tolerant rats and returned to control values by 24 hours after withdrawal In contrast, after nalorphine. precipitated withdrawal, Gunne and his associates (13) reported an increase in norepinephrine turnover in rat brain (which they attributed to stress). These differences in findings among the various studies of the effects of acute or chronic morphine administration or its withdrawal on the turnover of norepinephrine in brain may be related to differences in doses of drug, schedules and routes of administration, the techniques used to determine norepinephrine turnover, and the times these measurements were performed in relation to the schedule of drug administration.

Clinical Studios

Several investigators have examined the urinary excretion of catecholamines and metabolites during a cycle of morphine addiction and withdrawal in man (9,35). In one of these studies (involving two subjects) the urinary excretion of norepinephrine, epinephrine, dopamine, 3-methoxy-4-hydroxy-mandelic acid (VMA) normetanephrine (in one subject) and metanephrine (in one subject) increased at the beginning of the addiction phase when the dose of morphine was increasing. Tolerance appeared to develop to some of these changes (particularly the increase in epinephrine, norepinephrine and metanephrine excretion) during the course of continued morphine administration. During the withdrawal phase, excretion rates of all of these substances returned to near normal levels (35).

In another study (involving seven subjects), urinary excretion of norepinephrine and epinephrine, but not dopamine increased during the first two weeks of the addiction cycle when the dose of morphine was increasing. Initially, the increase in epinephrine excretion was greater than that of norepinephrine; however, epinephrine decreased during the latter part of the ascending dose phase. During the coarse of continued morphine administration (at a stable dose), tolerance was observed to the increase in epinephrine excretion -i.e., epinephrine did not differ from preaddiction values while norepinephrine tended to be higher than preaddiction levels but not as high as during the ascending dose phase. During withdrawal of morphine, urinary norepinephrine, epinephrine and dopamine levels were within the preaddiction range. Urinary epinephrine was elevated when examined 7 and 17 weeks after withdrawal (9). In a subsequent study, Hoeldtke and Martin (14) showed that these changes in urinary cate-

cholamines could not be ascribed to the alterations in urine volume that occurred during morphine administration and withdrawal.

The increase in urinary norepinephrine and epinephrine excretion during administration of ascending doses of morphine in human subjects is consistent with the results of comparable studies in animals (12,30). However, in contrast to the studies in animals that showed increased norepinephrine and epinephrine excretion during withdrawal of morphine (12,30) such increases were not observed during withdrawal in the clinical studies (9,35), possibly due to the lower doses and slower schedules of withdrawal.

In ongoing collaborative studies, we have been studying the urinary excretion of catecholamines and metabolites in human subjects participating in a series of experimental protocols that involved heroin administration. This paper reports the initial findings of these studies.

METHODS

In our initial biochemical study of the effects of heroin administration on Urinary catecholamines and metabolites, we examined these measures in nine subjects during an initial drug-free baseline period, a ten day period of heroin administration, and a subsequent period of methadone detoxification. All subjects in this study had a history of at least two years of heroin use with two documented treatment failures prior to study. The schedule of heroin administration allowed the patient to receive a maximum of 6 mg of heroin on Day 1, with an increase of 6 mg/day over each of the 10 days such that the maximum dose on Day 10 was 60 mg of heroin. Within each 24 hour period, the dosage of heroin was regulated according to a complex schedule that permitted the patient maximum freedom in choosing the schedule of administration, while at the same time preventing the patient from taking more than one quarter of the maximum daily dose in any six hour period. Throughout the 10 day period, virtually all patients administered the maximum dose each day, although the schedule of administration varied from subject to subject. After the tenth day of heroin administration, methadone was substituted for heroin. A large battery of psychiatric and behavioral assessments were performed during the course of this study, but these data will not be reported here.

This study was carried out in the context of a program designed to evaluate narcotic antagonist drugs in the rehabilitation of opiate addicts. The details of informed consent and citizen's review procedures involved in this work have been described elsewhere (19).

Throughout this study, daily 24-hour urine collections were obtained. Urinary catecholamines and metabolites* including norepinephrine, epinephrine, normetanephrine, metanephrine, 3-methoxy-4-hydroxymandelic acid (VMA), and 3-methoxy-4-hydroxy-phenlyglycol (MHPG) were determined (2,8,29,36) on the urine specimens obtained during the third and second day before heroin administration (Baseline), the day immediately before heroin administration (BH), the first three days of

** Urinary norepinephrine is thought to derive mainly from the peripheral sympathetic nervous system, while urinary epinephrine comes from the adrenal medulla. Urinary normetanephrine, the O-methylated metabolite of norepinephrine, is also thought to derive mainly from the peripheral sympathetic nervous system, and may best reflect the level of norepinephrine present extroneuronally and available to interact with receptors. Urinary metanephrine, the O-methylated metabolite of epinephrine, may provide a better index of the adrenal medullary output of epinephrine than does unchanged urinary epinephrine since the urine contains considerably more metanephrine than epinephrine. Urinary VMA, the deaminated O-methylated metabolite of both norepinephrine and epinephrine, is the major urinary metabolite of these catecholamines in man. Most VMA comes from peripheral sources and relatively 1ittle urinary VMA is thought to derive from the brain. Norepinephrine originating in the brain is largely excreted in the urine as the deaminated O-methylated metabolite MHPG, although some urinary MHPG may also come from peripheral sources. Although the exact fraction of urinary MHPG that derives from the brain remains problematic (with estimates in non-human primates ranging from 30% to 60%), it has been suggested that MHPG may be the urinary metabolite of norepinephrine that best reflects the synthesis and metabolism of norepinephrine in the brain (3,18,28).*

heroin administration (H1,H2,H3), the last three days of heroin administration (H8,H9,H10), and the first three days of methadone detoxification (D1,D2,D3).

In order to analyze the effects of heroin administration on these measures, change scores were computed. The values obtained on the third and second day before heroin administration (which were taken as the baseline values for each subject) were averaged; and for each subsequent day we computed individual change scores expressed as a percent of the average baseline value for each subject. From these individual change scores on each subject we then computed an overall mean value of the change score for each day for the entire group of nine subjects. Matched t-tests were used to determine the statistical significance of these daily change scores.

RESULTS

The mean baseline values for the group of nine subjects are shown in Table 1. The change scores for subsequent days are shown in Figure 1. Urine volume increased during the course of heroin administration, with statistically significant increases observed during the last three days of heroin administration and on the third day of methadone detoxification. In contrast to the increase in urine volume observed with heroin administration, creatinine excretion was not meaningfully altered during the period of heroin administration (Fig. 1).

TABLE 1

BASELINE VALUES FOR URINARY CATECHOLAMINES AND METABOLITES

Measure (Units)	Mean ± SEM
Urine Volume (ml/24hrs)	1134 ± 101
Creatinine (mg/24hrs)	1724 ± 93
Norepinephrine (µg/24hrs)	43 ± 6
Epinephrine (µg/24hrs)	9 ± 2
Normetanephrine (µg/24hrs)	312 ± 40
Metanephrine (µg/24hrs)	205 ± 22
VMA (µg/24hrs)	4113 ± 378
MBPG (µg/24hrs)	1817 ± 119

FIGURE 1

URINARY CATECHOLAMINES AND METABOLITES DURING HEROIN ADMINISTRATION

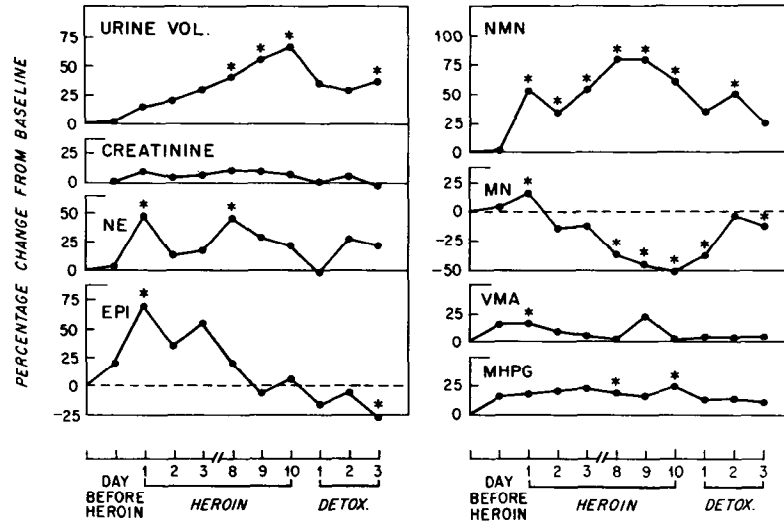


*Figure 1. Urinary catecholamines and metabolites during heroin admninistration. Baseline values for each measure were obtained by taking the average of the values for the third and second day before heroin administration. For each subsequent day, change scores expressed as a percent of the average baseline value for each of the nine subjects were computed. From these individual change scores the overall mean change score for each day for the entire group of nine subjects was computed. *p<0.05 (matched t-test).*

Figure 1 shows that all of the measures of urinary catecholamines and metabolites tended to be increased over baseline values on the first day of heroin administration, with the largest percentage increases occurring in noerpinephrine, epinephrine and normetanephrine. However, during the subsequent course of heroin administration, markedly different patterns of change emerged for each of these measures.

As shown in Figure 1, norepinephrine tended to be increased throughout the course of heroin administration. In contrast, epinephrine, which was increased during the early phase of heroin administration, returned to baseline values during the later period of heroin administration.

Normetanephrine excretion was markedly increased throughout the period of heroin administration. Highest values of normetanephrine occurred during the latter phase of heroin administration, and there was some return toward baseline values during methadone detoxification (Fig.1).

In contrast to this persistent increase in normetanephrine excretion, after the first day of heroin administration metanephrine excretion decreased with statistically significant decrements noted during the last three days of heroin administration. During methadone detoxification, metanephrine excretion returned toward baseline values (Fig.1).

After increasing on the first day of heroin administration, VMA returned to approximately baseline values (with the exception of an elevation on the ninth day of heroin administration that was not statistically significant and may be an artifact). Since VMA is the major deaminated-O-methylated metabolite of both norepinephrine and epinephrine in the periphery, the lack of change in VMA excretion during heroin administration may be explained by the finding that normetanephrine was increased but metanephrine was decreased (Fig. 1).

In these studies, we were particularly interested in the urinary excretion of MHPG, since (as noted above) an appreciable fraction of this metabolite may be derived from norepinephrine originating in the brain. As shown in Figure 1, MHPG excretion tended to be elevated throughout the course of heroin administration with statistically significant increases observed on Days 8 and 10. During methadone detoxification, there was a return toward baseline values.

Further analysis of the data on MHPG excretion (Fig. 21, indicated that a meaningful increase in MHPG excretion was not consistently observed in all of the subjects but only in a sub-group (four of the nine subjects). This is in contrast to the increase in normentanephrine excretion and the decrease in metanephrine excretion that was observed in the entire group of nine subjects. Moreover, it appeared

FIGURE 2

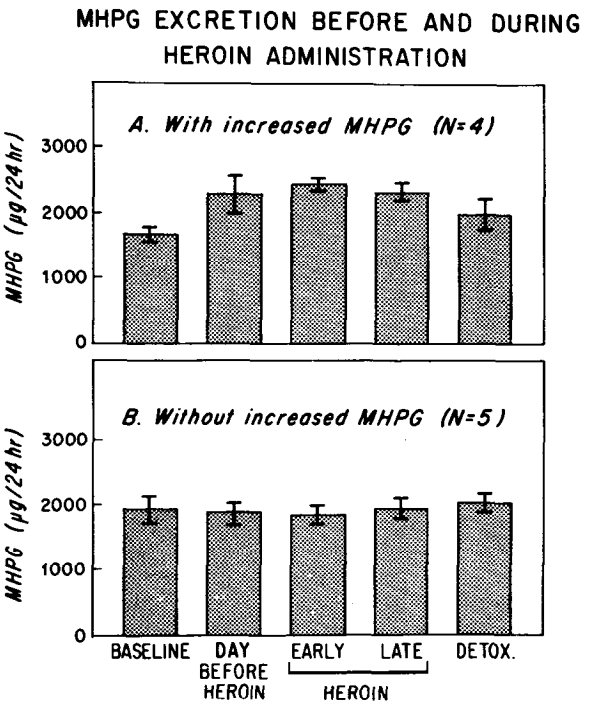


Figure 2. MHPG excretion before and during heroin administration in subjects with and without increased MHPG excretion during heroin administration. Individual values of MHPG excretion were averaged across subjects for each of the following periods: baseline (i.e., the third and second day before heroin administration); day before heroin; early heroin (i.e., the first three days of heroin a&inisration--H1,H2,H3); late heroin (i.e., the last three days of heroin administration--H8, H9,H10); and detoxification (i.e., the first 3 days of methadone detoxification).

as if the increase in MHPG excretion began on the day prior to the administration of heroin in the subgroup of patients with increased MHPG excretion during heroin administration (Fig. 2A).

DISCUSSION

In the present study of the effects of heroin administration on catecholamine metabolism in man, norepinephrine tended to be increased throughout heroin administration while epinephrine was increased above baseline only during the early phase of heroin administration. These findings are in agreement with the results of earlier studies (9,35) as reviewed above. The increase in urine volume observed during heroin administration in this study also agrees with earlier reports of increased urine volume during morphine administration (9,35).

The results of the present study showed a significant increase in urinary normetanephrine throughout heroin administration. Metanephrine which increased on the first day of heroin administration, decreased during the subsequent days of heroin administration. A decrease in phenylethanolamine-N-methyl-transferase (PNMT) activity in the adrenal gland of the rat has been described during chronic morphine administration (23), and such a decrease in adrenal PNMT activity might account for this decrease in metanephrine excretion during heroin administration.

During heroin administration, an increase in MHPG excretion was observed in a subgroup of four of the nine subjects studies. Since we have observed that tolerance develops to the morphine-induced increase in MHPG-SO_4 in rat brain, it is conceivable that persistence of, or development of, tolerance might account for the failure to observe an increase in MHPG excretion in all of the subjects.

As reported elsewhere physiological and behavioral data suggested that subjects in the subgroup with increased MHPG excretion during heroin administration manifested a greater response to injected heroin than did the remaining subjects (20). Three of the subjects in this subgroup elected to administer heroin more frequently, particularly in the latter phase of administration, and tended to have higher plasma-morphine levels. (However, the total amount of heroin administered, which was limited by the design of this study, was not different in this subgroup). All four of the subjects in this subgroup showed higher scores on measures of opiate intoxication and nodding behavior as well as the most pronounced respiratory depression (after each dose) particularly during the latter phase of heroin administration. Further studies will be required to confirm and extend these observations.

The observation that the increase in MHPG excretion began on the day prior to the administration of heroin, in the subgroup of patients with increased MHPG excretion during heroin administration, suggests the possibility of an anticipatory or conditioned response in these subjects, with the anticipation of heroin administration producing an increase in MHPG excretion. The subsequent administration of heroin in these subjects may then be required to maintain MHPG excretion at this level, and this might conceivably be related to the finding that these subjects elected to administer heroin more frequently. Further observations on a larger number of subjects will be required to confirm this suggestion of an anticipatory increase in MHPG excretion prior to heroin administration; and correlative studies in animals should help to determine whether opiate-induced increases in norepinephrine turnover (as reflected by MHPG levels) in brain can, in fact, be conditioned.

As described above, in our studies in animals, using levels of endogenous MHPG-SO_4 in brain as an index of norepinephrine turnover, we have found that one of the neuropharmacological effects of acute administration of morphine is to increase the turnover of norepinephrine in the brain (24,25). with continued administration of morphine, tolerance appears to develop to this effect on norepinephrine turnover and our findings further suggest that under these conditions the normal level of functioning of noradrenergic neuronal systems in brain may be dependent upon the continued administration of maintenance doses of morphine. Similar conclusions have been reported by Smith and his associates (31,32) who used a different technique to assess norepinephrine turnover in the brain.

Self-administration of morphine in animals can be attenuated by inhibition of norepinephrine biosynthesis (7). Thus, it is possible that the acute effects of morphine on norepinephrine turnover may help to account for the initial reinforcing properties of this drug, while its longterm effects on noradrenergic neurons may help to account for opiate-seeking behavior in addicted subjects. Further basic and clinical studies will be required to explore this possibility.

ACKNOWLEDGMENT

This work was supported, in part, by Grant No. DA 00257 from the National Institute on Drug Abuse.

REFERENCES

1. Ahtee, L. and I. Kaariainen. The effects of narcotic analgesics on the homovanillic acid content of rat nucleus caudatus. Eur. J. Pharmacol. 22: 206-208, 1973.

2. Anton, A.H. and D.F. Sayre. A study of the factors affecting the aluminum oxide-trihydroxyindole procedure for the analysis of catecholamines. J. Pharmacol. 138: 360-375, 1962.

3. Axelrod, J. Noradrenaline: fate and control of its biosynthesis. Science 173: 598-606, 1972.

4. Clouet, D.H. and M. Ratner. Catecholamine biosynthesis in brains of rats treated with morphine. Science 168: 854-855, 1970.

5. Clouet, D.H., J.C. Johnson, M. Ratner, M. Williams and G.J. Gold. The effect of morphine on rat brain catecholamines: turnover in vivo and uptake in isolate synaptosomes. In: Frontiers of Catecholamine Research, E. Usdin and S. Snyder, Eds., Pergamon, New York, pp. 1039-1042, 1973.

6. Costa, E., A. Carenzi, A. Guidotti and A. Revuelta. Narcotic analgesics and the regulation of neuronal catecholamine stores. In: Frontiers in Catecholamine Research, E. Usdin and S. Snyder, Eds., Pergamon, New York, pp. 1003-1010, 1973.

7. Davis, W. and S. Smith. Noradrenergic basis for reinforcement associated with morphine action in non-dependent rats. In: Drug Addiction Vol. 3, Neurobiologv and Influences on Behavuior, J.M. Singh and H. Lal, Eds., Symposia Specialists, Miami, 1974.

a. Dekirmenjian, H. and J.W. Maas. An improved procedure of 3-methoxy-4-hydroxyphenylglycol determination by gas-liquid chromatography. Anal. Biochem. 35: 113-122, 1970.

9. Eiseman, A.J., J.W. Sloan, W.R. Martin, D.R. Jasinski and J.W. Brooks. Catecholamine and 17-hydroxycorticosteroid excretion during a cycle of morphine dependence in man. J. Psychiat. Res; 7: 19-28, 1969.

10. Fukui, K. and H. Takagi. Effects of morphine on the cerebral contents of metabolites of dopamine in normal and tolerant mice: its possible relation to analgesic action. Brit. J. Pharmacol. 44: 45-51, 1972.

11. Gauchy, C., Y. Agid, J. Glowinski and A. Cheramy. Acute effects of morphine on dopamine synthesis and release and tyrosine metabolism in the rat striatum. Eur. J. Pharmacol. 22: 311-314, 1973.

12. Gunne, L.M. Catecholamines and 5-hydroxytryptamine in morphine tolerance and withdrawal. Acta Physiol. Scand. 58: Supplementum 204, 1963.

13. Gunne, L.M., J. Jonsson and K. Fuxe. Effects of morphine intoxication on brain catecholamine neurons. Eur. J. Pharmacol. 5: 338-342, 1969.

14. Hoeldtke, R.D. and W.R. Martin. Urine volume and catecholamine excretion. J. Lab. Clin. Med. 75: 166-174, 1970.

15. Johnson, J.C., M. Ratner, G.J. Gold and D.H. Clouet. Morpbhine effects on the levels and turnover of catecholamines in rat brain. Res. Comm. Chem. Path. Pharmacol. 9: 41-53, 1974.

16. Kuschinsky, K. and 0. Hornykiewicz. Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. Eur. J. Pharmacol. 26: 41-50, 1974.

17. Loh, H.H., R.J. Hitzemann and E.L. Way. Effects of acute morphine administration on the metabolism of brain catecholamines. Life Sci. 12: 33-41, 1973.

18. Maas, J.W., H. Dekirmenjian, D. Garver. D.E. Redmond. Jr., and H.D. Landis: Catecholamine metabolite excretion following intraventricular injection of 6-OH-dopamine. Brain. Res. 41: 507-511, 1971.

19. Meyer, R.E., Mirin, J.L. Altman and H.B. McNamee. Behavioral paradigm for the evaluation of narcotic antagonists. Arch. Gen. Psychiat. (in press).

20. Meyer, R.E., J.J. Schildkraut, S.M. Mirin, P.J. Orsulak, M. Randall, M. McDougle, E. Grab and P. Platz. Catecholamine excretion, behavior and mood in opiate addiction. In preparation.

21. Neal, M.F. Failure of morphine dependence in rats to influence brain noradrenlin turnover. J. Pharm. Pharmacol. 20: 950-953, 1968.

22. Puri, S.K., C. Reddy and H. Lal. Blockade of central dopamine receptors by morphine: effect of haloperidol, apomorphine and benztropine. Res. Comm. Chem. Path. Pharmacol. 5: 389-401, 1973.

23. Reis, D.J., P. Hess, E.C. Azmitia, Jr. Changes in enzymes subserving catecholamine metabolism in morphine tolerance and withdrawal in rat. Brain Res. 20: 304-312, 1970.

24. Hoffman, M., T. Reigle, P. Orsulak, J. Hardin and J.J. Schildkraut. Effects of morphine on the turnover of norepinephrine in rat brain. The Pharmacologist 16: 456 (ABS), 1974.

25. Roffman, M., T. Reigle, P. Orsulak and J.J. Schildkraut. The effects of acute and chronic morphine administration on the levels of 3-methoxy-4-hydroxyphenylglycol sulfate in rat brain. Res. Comm. Chem. Path. Pharmacol. 10: 403-417, 1975.

26. Rosenman, S.J. and C.B. Smith. ^{14}C-catecholamine synthesis in mouse brain during morphine withdrawal. Nature 240: 153-155, 1972.

27. Schanberg, S.M., J.J. Schildkraut, G.R. Breese and I.J. Kopin. Metabolism of normetanephrine-H. in rat brain: identification of conjugated 3-methoxy-4-hydroxyphenylglycol as the major metabolite. Biochem. Pharmacol. 17: 247-254, 1968.

28. Schildkraut, J.J., Neuropsychopharmacology and the Affective Disorders Little, Brown & Co., Boston, 1970.

29. Schildkraut, J.J. E.K. Gordon and J. Durell. Catecholamine metabolism in affective disorders I. Normetanephrine and VMA excretion in depressed Patients treated with imipramine. J. Psychiat. Res. 3: 213-228, 1965.

30. Sloan, J.W. and A.J. Eisenman. Long persisting changes in catecholamine metabolism following addiction to and withdrawal from morphine. Ass. Res. Nerv. Ment. Dis. 46: 96-105, 1968.

31. Smith, C.B., J.E. Villarreal, J.H. Bednarczyk and M.I. Sheldon. Tolerance to morphine-induced increases in ..C-catecholamine synthesis in mouse brain. Science 170: 1106-1107, 1970.

32. Smith, C.B., M.I. Sheldon, J.H. Bednarczyk and J.E. Villareal. Morphine-induced increase in the incorporation of ..C-dopamine and ..C-norepinephrine in the mouse brain: antagonism by naloxone and tolerance. J. Pharmacol. Exp. Ther. 180: 547-557, 1972.

33. Sugrue, M.F. The effects of acutely administered analgesics on the turnover of noradrenlin and dopamine in various regions of the rat brain. Brit. J. Pharmacol. 52: 159-165, 1974.

34. Vogt, M. The concentration of sympathin in different parts of the central nervous system under normal conditions and after the administration of drugs. J. Physiol. 123: 451-481, 1954.

35. Weil-Malherbe, H., E.R.B. Smith, A.J. Eisenman and H.F. Fraser. Plasma catecholamine levels and urinary excretion of catecholamines and metabolites in two human subjects during a cycle of morphine addiction and withdrawal. Biochem. Pharmacol. 14: 1621-1633, 1965.

36. Wilk, S., S.E. Gitlow, M. Mendlowitz, N.J. Franklin, H.E. Carr and D.D. Clark. A quantitative assay for vanillylmandelic acid (VMA) by gas-liquid chromatography. Anal. Biochem. 13: 544-551, 1965.

AUTHORS

Joseph J. Schildkraut, M.D., Roger E. Meyer, M.D., Paul J. Orsulak, Ph.D., Steven M. Mirin, M.D., Mark Hoffman, Ph.D., Patricia A. Platz, B.S., Edwin Grab, A.M., Mary *E.* Randall, M.A., Mark McDougle, B.A.

Neuropsychopharmacology Laboratory, Massachusetts Mental Health Center, Boston, Massachusetts 02115;

Alcohol and Drug Abuse Research Center, McLean Hospital, Belmont, Massachusetts 02178; and

Department of Psychiatry, Harvard Medical School, Boston, Massachusetts 02115

CONCLUSION

The purpose of this monograph was to bring together in a single volume papers describing different lines of current research in the area of brain amine functioning. Such a broad approach is necessary for the evaluation of the entire state of the art at this time.

Several conclusions derive from these papers. First, there ore several hypotheses of behavior, not all of which are complementary or consistent. Secondly, these hypotheses vary greatly with regard to presently available supporting data and the breadth of behavior each hypothesis encompasses. Thirdly, with only a few exceptions, these theoretical constructs concerning the roles of the brain monoamines are based upon correlations obtained from highly specific experimental manipulations, the results of which are difficult to generalize to other aminergic research.

The problem, as suggested in the Introduction and several of these papers, probably lies in some assumptions until recently underlying aminergic research and the techniques being employed. For example, it appears doubtful that results of experiments measuring whole-brain or even large section amine levels can in any functional way be related to alterations in specific behavioral syndromes in anything but the crudest manner. Futhermore, there exists a basic problem with the dependent variables we seek to measure. Variables such as changes in brain amine levels, ratios or turnover rates even in very limited brain areas, may in fact have little functional relationship to the behaviors under investigation. Additional basic research is required to determine which measurements are reliable and valid in terms of the functioning of brain neuronal systems. New approaches to the problem, new methodologies and even entirely new questions are needed.

The advent of new dissection and separation techniques together with more sensitive analytical tools such as the gas chromatograph-mass spectorscopy combination may provide some of these much needed answers. Other techniques such as the electrical identification of single cell units which selectively fire in response to highly specific visual stimuli but not to other visual stimulation, the use of an imunological approach to selectively bind hypothesized receptors in

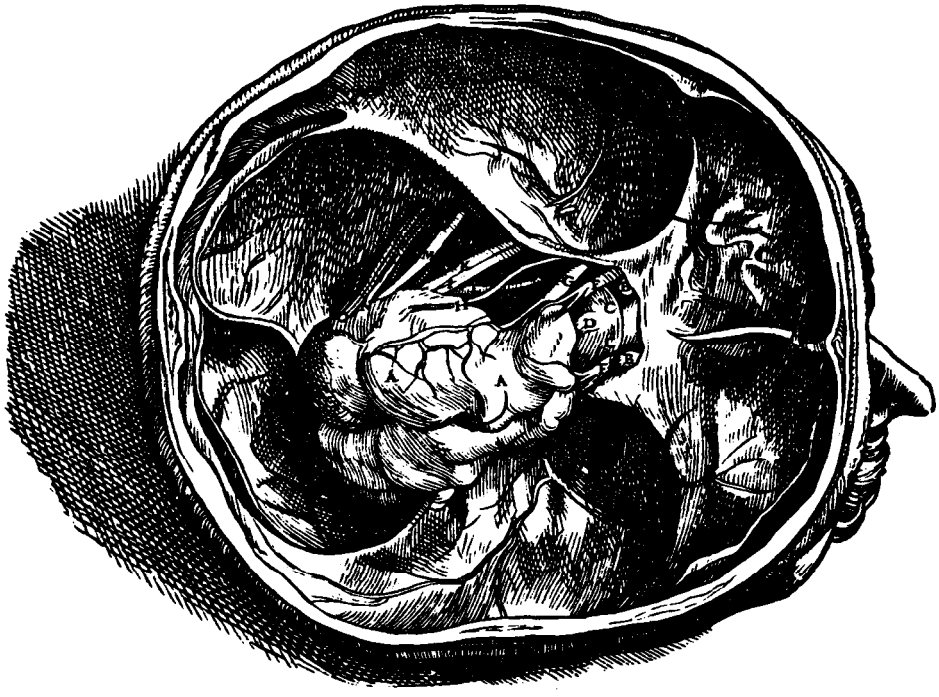
specific brain regions and a number of as yet untried approaches, may aid in providing the needed impetus to research in this area.

As our techniques become increasingly refined and new methodologies and approaches are employed, our understanding of functional neuronal dynamics will increase. With this will come on increased ability to quantify and evaluate the relevance of a multiplicity of dependent variables with more precision. As that occurs, so will our ability increase to see relationships across behaviors and ultimately our ability to generalize from them. Thus, by a kind of paradox must we progress through ever greater intellectual and technical specificity in order to achieve greater conceptual generality.

This publication was not meant to be comprehensive. There are additional areas of amine research which are not covered in this volume. Investigations into the biochemical mechanisms of alcoholism, narcotic addiction and drug abuse have significant social importance. Yet, advances in these fields hove also been hindered by the above mentioned problems.

It is my hope that the questioning put forth in this monograph, the growing concern of my fellow researchers for the generalizability of their work, the new approaches to these problems and the greater sophistication of older techniques, together with an increased scientific and social impetus to understand and deal with these areas, will culminate in a renewed and sustained drive towards understanding the workings of the central nervous system and in particular the functioning of the brain monoamines.

Bruce K. Bernard
Storrs, Connecticut
1975



☆ U. S. GOVERNMENT PRINTING OFFICE : 1975 632-513/1084

DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE

ALCOHOL, DRUG ABUSE, AND
MENTAL HEALTH ADMINISTRATION
5600 FISHERS LANE
ROCKVILLE, MARYLAND 20852

OFFICIAL BUSINESS
Penalty for private use, $300

POSTAGE AND FEES PAID
U.S, DEPARTMENT OF H.E.W
HEW 389

NOTICE OF MAILING CHANGE

☐ Check here if you wish to discontinue receiving this type of publication.

☐ Check here if your address has changed and you wish to continue receiving this type of publication. (Be sure to furnish your complete address including zip code.)

Tear off cover with address label still affixed and send to:

Alcohol, Drug Abuse, and Mental Health Administration
Printing and Publications Management Section
5600 Fishers Lane (Rm. 6-105)
Rockville, Maryland 20852

DHEW Publication No. (ADM) 76-295
Printed 1975

U.S. DEPARTMENT OF HEALTH. EDUCATION. AND WELFARE
PUBLIC HEALTH SERVICE
ALCOHOL, DRUG ABUSE, AND MENTAL HEALTH ADMINISTRATION