THE EFFECTS OF EXERCISE UPON RAT BRAIN CATECHOLAMINES

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
BARRY S. BROWN
1970

This is to certify that the

thesis entitled

THE EFFECTS OF EXERCISE UPON RAT BRAIN CATECHOLAMINES

presented by

Barry S. Brown

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Physical Education

Major professor

Date 7/29/70

O-169

ABSTRACT

THE EFFECTS OF EXERCISE UPON RAT
BRAIN CATECHOLAMINES

By

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Brain catecholamine concentrations and depletion rates were investigated in male albino rats to observe the alterations accompanying a specific "middle-distance interval-training program" of eight-weeks duration. This program was intended to simulate the type of training used by man for the middle distance running events (i.e., 880-yard or one-mile run).

Eighty adult, male, Sprague-Dawley rats were randomly divided into sedentary and exercise treatment groups. An electronically controlled, self-propelled, running wheel was utilized to train the exercise group in a progressive middle-distance interval regimen one hour per day five days per week.

Two days following completion of the training program, the sedentary and exercise groups were each randomly divided into three subgroups and subjected to final treatment protocol as follows:

Final Treatment Subgroups

E = Trained rat run through "normal" exercise routine

E_{wh} = Trained rat placed in exercise wheel secured to prevent rotation

E_{sed} = Trained rat placed in sedentary cage

S_{ch} = Sedentary rat placed in "cheerleader" cage (rat receives same amount of shock as runner, E, but cannot escape)

S_{wh} = Sedentary rat placed in exercise wheel secured to prevent rotation

S_{sed} = Sedentary rat placed in sedentary cage

One and one-half hours prior to the final treatment (and 2 1/2 hours before sacrifice), each of the subgroups was then randomly divided into two equal parts. One-half of the animals in each group were injected with distilled water and the other half with alpha-methyltyrosine. The latter drug prevents synthesis of catecholamines by competitively inhibiting tyrosine hydroxylase.

Animals were sacrificed by decapitation and their brains were removed and quick frozen in chilled isopentane. Catecholamines were extracted with perchloric acid, adsorbed onto alumina, eluted and analyzed using standard fluorometric procedures.

The absolute weights of brain and heart of the sedentary and exercise rats did not differ significantly. When expressed as per cent body weight these organs were significantly heavier in the trained rats.

Motor activity, measured by total revolutions run in the exercise wheel during final treatment, was not significantly different between runners receiving distilled water and those injected with alpha-methyltyrosine.

Higher brain norepinephrine concentrations ($p < .05$) were found in the trained compared to sedentary rats. Dopamine concentrations were not significantly different. The increased brain norepinephrine concentration found in the trained rats may have been caused by the continuous demand placed upon the sympathetic nervous system by the exercise.

Exercise and/or shock among trained rats potentiated alpha-methyltyrosine induced depletion of brain norepinephrine. The lack of dopamine depletion among trained rats following exercise and alpha-methyltyrosine suggests conservation of this amine in the performance of running exercise.

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Physical Education
College of Education

1970

Dedicated to my cherished wife, Gail,
daughter, Sherry, forthcoming son,
Mom and Dad

ACKNOWLEDGMENTS

A special word of appreciation is extended to those individuals most instrumental in the formation, constructive criticism and completion of this study. Dr. Wayne Van Huss, my major advisor, supplied the initial impetus, needed encouragement and continued dedication that served as the motivational drive toward the accomplishment of the task. The facilities for biochemical analyses of brain tissue were generously supplied by Dr. Kenneth Moore, in spite of the lack of available space. Dr. Moore provided corrective criticism when the need arose. Mrs. Mirdza Gramatins deserves special mention for her patience during the initial phases of the analysis.

Voluntary assistance from Messrs. Abe Albenda and George Janes during sacrifice protocol involved many arduous hours, for which I am grateful. Without the assistance of these two gentlemen, results could not have been attained.

Training facilities for the rats were provided by Dr. William Heusner, who found it necessary to schedule other projects around my time slot.

Deepest appreciation is expressed to my wife Gail, whose patience in typing the original manuscript was limitless, and whose encouragement during times of stress was met with scowls and abusive remarks by her husband.

Final sentiment lie among the ashes of the most cooperative subjects any researcher could ever hope to assemble: rattus rattus.

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LIST OF ABBREVIATIONS

a-MT or alpha-MT	alpha-methyltyrosine
CA	catecholamine(s)
CAR	conditioned avoidance response
CDS	cumulative duration of shock
DM	dopamine
DM-B-Hydroxylase	dopamine-beta-hydroxylase
E	epinephrine
NE	norepinephrine
PER	per cent expected revolutions
TER	total expected revolutions
TRR	total revolutions run

CHAPTER I

INTRODUCTION

A large body of literature has accumulated over the years demonstrating changes in the cardiovascular, cardiorespiratory and muscular systems as a result of chronic exercise. Most of the studies, however, can be interpreted only in generalities because the exercise stress utilized has not been clearly defined. Little is known concerning either the differential effects of specific exercise regiments or the mechanisms underlying the anatomic and physiologic changes which are produced by such regimens.

An area of investigation of particular interest was the effects of exercise upon brain catecholamine (CA) concentration. Single bouts of intense swimming had been found to yield brain norepinephrine (NE) levels below control values [67]. An increase in the synthesis rate of brain NE was observed in rats exercised on a treadmill [38]. In discussing the results of these investigations the authors expressed the belief that brain catecholamines are replaced during physical activity and may be involved in the central control of the autonomic nervous system. Arguments have been presented implicating NE and/or dopamine

(DM) as transmitters of neuronal networks that modulate: (a) central regulation of the sympathetic nervous system [12, 44], (b) states of alertness and motor activity [16], (c) emotional affective states [87], and (d) temperature regulation [4, 52]. On the basis of these arguments and partial evidence [38, 68] it is reasonable to postulate that brain catecholamines may be involved in the central response to a long-term exercise regimen.

A dearth of evidence exists concerning both the differential effects of specific exercise regimens and the chronic effects of such regimens upon neurochemical correlates (CA in particular) in the brain. Since a need exists for quantitative evidence of this nature, it was decided to study the effects of a long-term, defined, exercise regimen upon brain CA levels.

Statement of the Problem

1. To determine the changes in catecholamine content occurring in the brain of male albino rats following eight weeks of "middle-distance, interval-training," The training program was intended to simulate a middle distance (880-yard or one mile) running regimen.

2. To determine the relationship of various organ weight measures and performance criteria to brain catecholamine concentrations of sedentary and exercised rats.

Rationale

Specific physical changes resulting from exercise programs may be influenced by or exert fine control over central nervous system components. Measurable components which might provide further insight into these mechanisms were judged to be the NE and DM levels of the brain. It was reasoned that, following the training program of eight weeks, if half of the animals in each of the groups were injected with a catecholamine synthesis inhibitor, alpha-methyltyrosine (a-MT, 93), and the other half with distilled water, under varying final treatment conditions (including exercise) the degree of catecholamine synthesis attributable to exercise could be estimated. The study was designed around this concept, including appropriate subgroups to provide for controls.

Limitations of the Study

1. Whole brain catecholamine analysis may mask significant changes taking place in smaller areas of the brain (e.g., NE in the hypothalamus and DM in the extrapyramidal tract).

2. Validity of the electronically controlled running wheel in producing a physically trained animal is lacking. Adaptation of rats to shock may be contributing to the changes which are attributed to the chronic effects of exercise.

3. Temperature control was not instituted for the first two shipments of animals due to financial limitations.

4. The inability to maintain shock control animals alongside trained rats for the duration of the regimen prevented direct evaluation of the contribution of shock to the training effects produced by the exercise program.

CHAPTER II

REVIEW OF THE LITERATURE

A review of literature encompassing the past twenty years has failed to uncover any studies undertaken to assess the effects of long-term exposure to exercise upon brain catecholamines. The effects of differential physical activity upon possible central neurotransmitter substances remains unexplored. The focus of the following literature review lies in the area of brain CA analyses that have been undertaken within the past twenty years to elucidate the role of central amines under conditions of stress.

Exercise vs Trained Condition

A popular misuse of the word exercise requires clarification, just as the nebulous term drug places burden upon the author to describe with greater precision the treatment employed. Exercise, per se, is neither specific nor informative of the intensity or duration of the physical stress employed. Physical work can be performed in many ways under many conditions for varying lengths of time. A complete description of training procedures is necessary, if one wishes to apply the results of an exercise regimen to

similar populations. Effects of swimming exercise may not be applicable to the same species (under identical environmental and dietary control) forced to run in a rotating drum. Neither can one generalize results after one bout of severe physical activity to the effects of exercise; rather, attention should be drawn to the acute effects of a specifically defined activity upon previously sedentary, individual (or multiple) housed animals.

Swimming procedures have been utilized by a number of authors [4, 9, 30, 31, 40, 68] to demonstrate short-term effects of exercise upon brain CA. All investigators neglected to specify in their conclusions the application of their results to acute effects of swimming. Moreover, it has been shown that swimming as an exercise stressor cannot be controlled [21, 61].

Spontaneous motor activity has been determined with devices ranging from a lever-pressing apparatus [39] to actophotometers measuring movement in terms of recorded changes in foot position [63, 98] and to a variety of voluntary running wheels recording number of revolutions run per unit time [13, 23, 65, 78, 79, 82, 83].

The treadmill was used as a training device in four instances by authors investigating CA changes following exercise [10, 22, 42, 91], with only one author [22] giving full specifications of the training program. A more detailed description of the exercise protocol was presented

by authors utilizing a motorized drum [2, 5, 42, 96]. However, five sources failed to mention the procedure employed [29, 58, 59, 62, 71]. Treadmill exercise of rats, in general, is a poor technique as only about 50% will run without extensive training [41].

Failure to specify exercise procedures may stem from the authors' use of exercise as a vehicle to stimulate various areas of the central nervous system, especially the central mechanism controlling sympathetic function. It may be argued, chemically speaking, that all forms of stress (physical or otherwise) evoke the same central pattern of response. This viewpoint cannot be accepted as there was a marked disparity in the brain CA response to each of the activities outlined above. These differences are discussed in the sections which follow.

Effects of Stress Upon the Brain

Brain Weight and Stress

The effects of exercise upon rat brain weight have been investigated following three to six months of voluntary, running activity [25, 26, 43]. A small (4%) but consistent increase in relative brain weight was observed by these authors. The data were not statistically analyzed; therefore, results must be considered speculative. Swimming rats to exhaustion (15 to 30 minutes in 15°C water or 4-6 hours in 23°C water) had no effect on brain weight [4].

Reduction of the environmental temperature to 5-7°C for three to five months [66] and isolation or community housing [65] of female rats for 15-17 weeks also failed to alter brain weight significantly. Socially impoverished and environmentally enriched housing conditions had no effect upon total rat brain weight [51]. However, a breakdown of the various areas of the brain showed that enriched rats possessed increased cortical and decreased subcortical weight. In addition to increased cortical weight, a series of cortical changes were observed among enriched rats including: (1) higher cortical ratio of cholinesterase to acetylcholinterase (Che:AChe), (2) larger capillary diameters, and (3) increased number of glial cells [88].

Brain CA Concentration and Stress

The application of electric shock to the foot pads of rats has been used as an acute stress to lower brain CA stores [9, 47, 53, 60, 68, 77]. However, Thierry et al. [95] did not observe any change in rat brain NE levels following three hours of intermittent shock at 0.8 ma; and Iwamoto and Sato [47] obtained an increase in brain NE after four hours of grid shock. The latter authors suggest the rise in brain NE was due to tension and apprehension provoked by recurrence of the shock rather than the actual pain.

Depletion of brain NE due to immobilization observed by Bliss and Zwanziger [9] was not confirmed under similar

restraint stress by other authors [14, 99, 101]. Indeed, Welch and Welch [99, 101] reported increased CA concentration in brains of mice made hyperexcitable by eight to twelve weeks of isolation. They suggested the higher levels of brain NE observed in isolated mice may have been due to an increased sensitivity of NE receptors as a result of slower NE release [100].

Sustained swimming for four hours in 23°C water [4, 68] and a survival swim in ice water [40] caused significant decreases in rat brain NE concentration. Both Hamburg [40] and Barchas and Freedman [4] stated that decreased NE levels correlated with behavioral depression following the swim, suggesting that brain NE is important in the behavioral response to stress. Decreased CA levels signify for the most part, increased utilization of CA during stress, such that the stressor applied evokes neural stimulation capable of releasing amine stores more quickly than they can be replenished.

Sedentary rats exposed to treadmill exercise of one [38] and three [4] hours duration failed to show any change in brain NE concentration. Maintained levels of brain CA indicate that synthesis of new amine kept pace with the increased CA depletion resulting from the flow of neural impulse.

Regulation of Brain CA Concentration During Stress

The control of endogenous CA levels following stress must be mediated through a feedback mechanism designed to regulate metabolic degradation, physiological inactivation and/or synthesis of the transmitter substance. Intraneuronal CA is normally metabolically inactivated by deamination [34, 48]. Destruction of endogenous CA stores following stress may be prevented from altering MAO activity, thereby decreasing the normal amount of deaminated metabolites formed [33, 54, 60, 101, 102]. Catecholamines would be conserved by this mechanism, maintaining adequate amine stores to be utilized during stress.

Active reuptake of physiologically released amine during stress may be necessary to maintain pre-stress levels of endogenous CA [8]. This view is supported by the observations of increased uptake of labelled NE in the rat heart following repetitive stimulation of the cervical sympathetic trunk [17] and cold exposure [32].

Brain CA Synthesis and Stress

The role that synthesis must play to supply newly formed intraneuronal amine following stress depends upon the degree to which nerve endings have been depleted of its CA stores. Synthesis plays a greater role than uptake in the maintenance of NE following nerve stimulation of the guinea pig hypogastric nerve-vas deferens preparation [1].

Three hours of intermittent shock [11] and one hour of cold [36, 38] increased the turnover rate of labelled NE in the rat brain. Running exercise on a treadmill for one hour caused an increased synthesis rate of NE (0.06 ug NE/g brain/hour) in the rat brain [38]. This evidence supports the concept that the synthesis rate of rat brain CA increases during stress.

Control of CA Synthesis

The formation of CA normally proceeds in the following order: tyrosine ⟶ dopa ⟶ DM ⟶ NE. The mechanism underlying control of CA synthesis was elucidated following the discovery of tyrosine hydroxylase [72, 97]. This enzyme was found to be the rate-limiting step in CA formation, catalyzing the conversion of tyrosine to dopa. The presence of free (nonbound) CA is believed to act as its own end-product inhibitor by inactivating a tyrosine hydroxylase cofactor, affecting tyrosine transport into the cell, or activating other endogenous inhibitors [1, 88]. The end result is to maintain the concentration of functional CA at a near constant level. Release of brain CA following stimulation of appropriate central neurons decreases the content of functional amine. End-product inhibition at tyrosine hydroxylase is, thereby, removed and conversion of tyrosine to dopa proceeds at a faster rate [34, 37]. However, actual levels of tyrosine hydroxylase do not increase following stimulation of the rat submaxillary

gland [88] and heart [37]. These authors suggested that NE decreases the rate of synthesis by combining with tyrosine hydroxylase, rather than by altering its levels. A comprehensive summary of CA metabolism and control of amine synthesis can be found in a recent review [34].

"Functional vs Bound" Storage of CA

Incomplete depletion of brain amine stores following total behavioral depression has prompted investigation of the presence of a small functionally active store of brain CA.

A number of authors have used tyramine to deplete stores of NE in the rat heart. In heart tissue, there was believed to be a tyramine-resistant and a tyramine-releasable pool of NE [35, 49, 50, 80, 81]. This condition created a biphasic decline of non-tracer NE because of incomplete availability of tyramine. Constant infusion of tyramine reduced heart NE stores at a single exponential rate. If infusion is not sustained, tyramine became metabolically inactivated. Based upon information gathered from uptake studies of labelled NE [74, 92] and various CA releasing agents [18, 73], a model of intraneuronal NE storage was proposed. Two or more pools of NE were described; one pool is filled with nonbound "free" NE capable of being bound onto receptor sites at synaptic endings. This represents approximately 10% of the total NE stores. The other pool contains one or more nondiffusable complexes

of NE in granules [89, 94], requiring ATP and MG^{++} [34] for binding. Inhibition of CA synthesis showed that these pools are in ready equilibrium and during such inhibition amine stores decline at a single exponential rate [18, 73, 78]. The existence of a small functionally active store of brain CA has been used to account for the behavioral lag of synthesis inhibitors following prior depletion of CA stores [14, 28, 64].

Interaction of Alpha-MT with Drugs and Stress

Alpha-MT has been used to deplete CA stores in various organs and to calculate synthesis rates following exposure to a variety of stressors. The procedure is based upon a single rate of decline of CA disappearance subsequent to drug application [20] and assumes: (1) "a-MT totally blocks tyrosine hydroxylase with a sustained effect, (2) a-MT should not release NE or DM, not affect its metabolism, and (3) no stores of dopa or DM must remain in the neuron for possible conversion to NE following a-MT."

The advantage of calculating synthesis rate (based upon a-MT-induced CA decline) compared to measurement of steady-state values is readily evident because endogenous CA levels may not change subsequent to amine release if synthesis is not prevented from replenishing CA stores. Stress may not affect steady-state values but may increase the synthesis rate of brain CA. Measurement of steady-state

values only would erroneously indicate that the stress was ineffective in evoking a central adrenergic response.

A comparison of techniques for determining CA synthesis rates shows no statistically significant difference between use of a-MT and measurement of specific activity following administration of labelled amines [45, 46]. The relative ease of the a-MT procedure, and limited budget facing many researchers, have prompted wide-spread adoption of the first method.

Alpha-MT administered to rats placed in a cold room for several hours prevented body temperature regulation after a lapse of two hours and depleted brain NE and DM completely [19]. They concluded brain CA is essential to elicit shivering, which, in turn, is necessary to utilize energy substrates mobilized by the peripheral NE still present. Rats exposed to cold (4°C) adapted within seven days and resumed normal synthesis rate of brain NE (DM was not affected) as measured by steady-state kinetics following use of a-MT [90]. It appears that brain NE can be modified subsequent to chronic exposure to environmental stress.

Rech et al. [85], Dominic and Moore [24], Moore and Rech [70, 71[and Pirch et al. [79] examined the effects of a-MT upon various forms of behavioral response (shuttle box for CAR; rotarod for muscle tone and coordination; locomotor activity on a revolving drum to indicate an "unconditioned behavioral response to a non-specific stimulus").

In general, results equate a-MT depletion of brain CA levels with the onset and duration of behavioral depression. Rech et al. [83] hastened the onset and enhanced the intensity of behavioral depression due to a-MT by pretreating animals with reserpine. This procedure exhausted reserve stores of amine and demonstrated that only a small, readily available pool of mediator is necessary for normal function.

Measurements of motor activity determined in actophotometric units by Weissman et al. [98] indicated that behavioral effects of a-MT are correlated with the in vivo inhibition of tyrosine hydroxylase, rather than levels of NE. This is in agreement with previous reviews that assume NE is released from a small "functional pool" accounting for the observed behavioral depression.

Spector et al. [93] produced mild sedation and impaired motor activity in cats and guinea pigs by administration of a-MT. They are generally considered responsible for the discovery of the inhibiting effect of this compound upon tyrosine hydroxylase.

The acute effects of running exercise (at 1 ft/sec) upon rat brain CA concentration were investigated by Gordon et al. [38]. They discovered that one hour of running did not significantly lower NE stores, but enhanced the depletion of brain NE (DM was not affected) by administration of a-MT (200 mg/kg) one-half hour prior to exercise. This implies the rate of brain NE synthesis was increased during

exercise even though steady-state levels (ug/g) remained unchanged.

Chronic Effects of Exercise Upon CA

A void exists in the literature concerning the adaptation of brain catecholamines to chronic exercise regimens.

Three days of grid shock at 1 milliampere for one minute per day caused an increase in rat brain NE levels in the frontal pole and caudate nucleus four days following cessation of the treatment [76]. Nielson and Fleming observed, in addition, increased DM in the caudate nucleus after three days of cold stress (daily immersion for ninety seconds in 1-2°C ice water).

Thierry et al. [95] increased rat brain NE by 22% after three days of intermittent shock at 0.8 milliamperes (followed by one day of rest before sacrifice). These "stress adapted" animals displayed increased NE turnover when subjected to shock immediately prior to sacrifice.

In view of the training procedures utilized in the present study, the "chronic" effects of shock stress reviewed above [76, 95] may have been applicable to the exercise regimen employed.

CHAPTER III

EXPERIMENTAL PROCEDURE

The present study was undertaken to determine the chronic effects of a specific exercise regimen upon rat brain CA concentration. A secondary objective was to determine the contributory effects of shock, exercise and a-MT upon per cent CA depletion in the brain.

Overview of the Experimental Design

The design is illustrated in Figure 1 and first presented briefly for perspective. The animals were randomly divided into sedentary and exercise groups. The exercise group was placed on a middle-distance interval-training program intended to simulate middle distance running (880-yard and one mile run) in man. At the end of the training program the sedentary and exercise groups were each randomly divided into three subgroups for the final treatment: sedentary, wheel secured, and exercise (shock control in the sedentary group). Just prior to the final treatment one-half of the animals in all subgroups were randomly selected for injection with the catecholamine synthesis inhibitor a-MT. The other half was injected with distilled

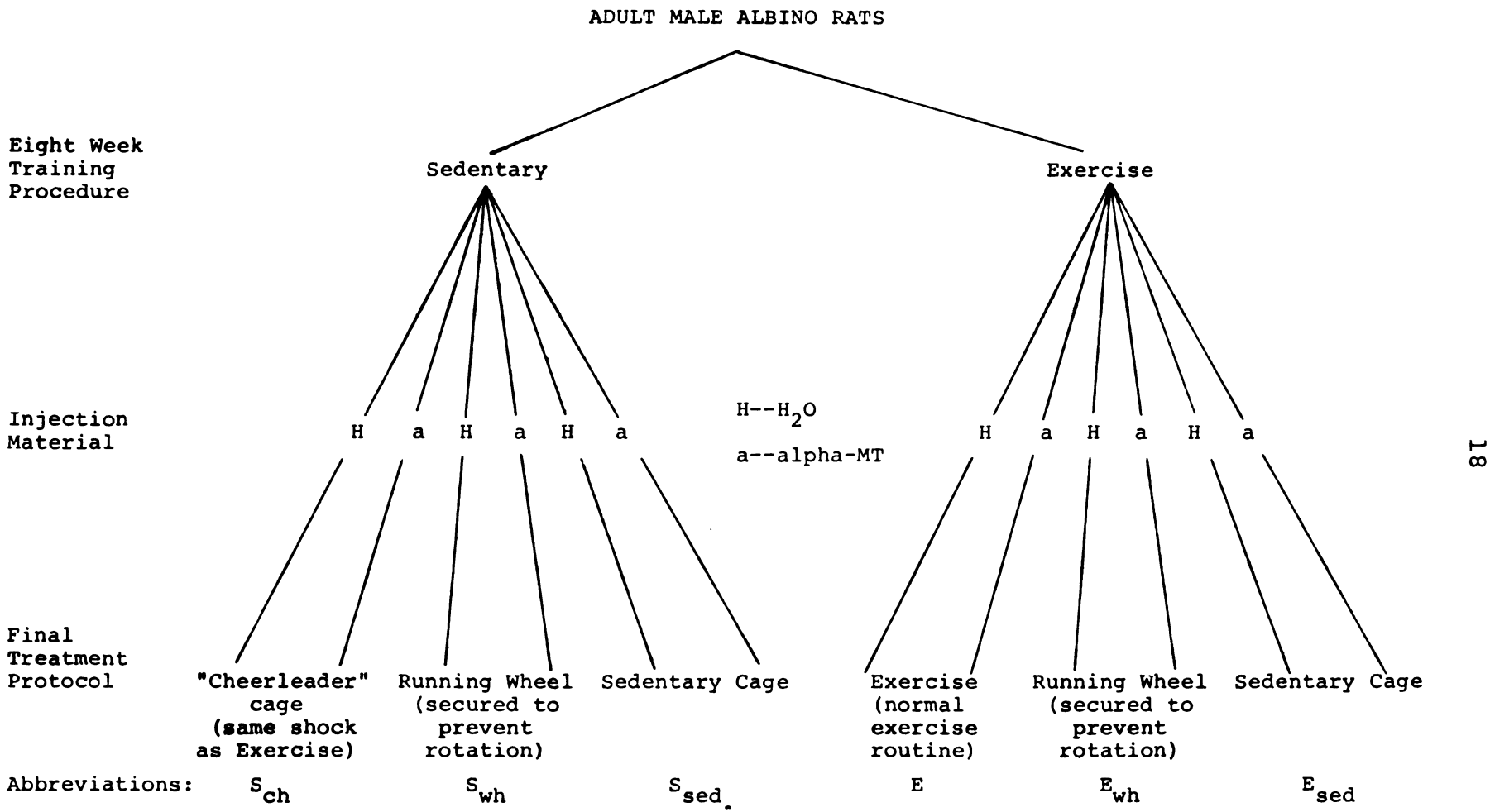

Figure 1: Experimental design of treatment groups during the eight week training procedure and for the final treatment. Injection material administered 1 1/2 hours prior to the final treatment and 2 1/2 hours prior to sacrifice.

water. Two and one-half hours following injection all animals were sacrificed. The brains were removed, quick frozen and subsequently analyzed fluorometrically for CA concentration. The logic of this design was to determine the chronic effects of the exercise program upon CA concentration with controls for the electric shock received and animal placement in the running wheel which were considered to be possible distorting factors. The use of a-MT as a CA synthesis inhibitor in half of the animals of all groups then permitted a quantitative comparison of CA depletion against controls under all of the experimental conditions.

The detailed account of the experimental procedures which follows includes: receiving and animal assignment to groups, routine protocol in handling of the animals, final treatment protocol with a detailed description of each final treatment subgroup, the biochemical techniques, and the statistical analyses.

Receipt and Assignment of Animals

Three shipments of adult (75 day old), male, Sprague-Dawley albino rats, in lots of thirty, thirty and thirty-eight, respectively, were received in prearranged stagger approximately three months apart. Upon receipt, each rat was transferred to an individual wire-meshed housing unit, 8" x 8" x 10", with free access to an adjacent activity wheel. Mechanical revolution counters provided with each activity wheel allowed individual activity records to be

kept daily for each rat. This procedure provided the basis for exclusion of a total of six rats per shipment, three on each end of the activity scale, based upon a daily average over a two-week period. The remaining eighty animals were randomly assigned to one of two experimental treatments, sedentary or exercise. Both groups were placed in individual housing units, but without access to the activity wheels, and received identical treatments with the exception of the experimental variable.

Routine Animal Care Procedures

During the two-week pre-training acclimation and eight-week training regimen, all rats were subjected to similar environmental stimuli. Animals were handled daily with a gentle approach to avoid overt and aggressive behavior evidenced in rats living under isolation and environmental impoverishment [51]. Daily body weights were taken and recorded to the nearest gram. Water and Wayne Lab Blocks were provided to each animal ad libitum, and notes were made concerning the general health and appearance using skin and fur texture, eye color and nasal and oral mucous secretions as criteria.

All cages were steam cleaned weekly and temperature control was maintained between 70-75°F, whenever possible. The handling of animals was restricted to one technician and all other laboratory personnel were excluded from entering the animal quarters.

Training Regimen

The training procedure employed for the exercise treatment group was designed to subject each rat to a moderately intense and fairly specific exercise regimen. It was essential to choose an activity not only within the physiological capacity of the animal, but one which involved the performance of physical skills normally encountered by this species.

The decision was made to utilize a recently developed electronically controlled running wheel employing the principles of behavioral response to initiate physical activity by the rat.[1] Each exercised rat was placed in the running wheel one week prior to the start of training and allowed to run in the apparatus at will. Three days prior to the first recorded session, rats were conditioned to respond to a light stimulus by running to avoid an electric shock of 1.2 milliamperes.

Electronic controls were preset dictating: (1) the speed (ft/sec) at which the rats had to run to avoid the unconditioned stimulus (shock); (2) the time required in each work period (sec); (3) the time during which the rat was allowed to rest (controlled by an automatic brake system), (4) the amount of time the animal had to accelerate

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[1]The electronically controlled running wheel was designed and developed at the Human Energy Research Laboratory, Michigan State University, by Dr. W. W. Heusner and Robert Wells.

at the conclusion of the rest period (after the brake system is released) to avoid shock. At the start of each work cycle, the rat was again conditioned to avoid shock by responding to the light stimulus. This work-rest cycle repeated itself until a preset number of repetitions was attained, at which time the rat was allowed a five-minute rest period, completing one bout. A number of bouts were automatically repeated until conclusion of the daily program.

The number of revolutions covered during the daily program, referred to as total revolutions run (TRR), was obtained from an electromechanical counter attached to each wheel and recorded on daily record sheets along with the cumulative duration of shock (CDS) during the work periods. Additional measurements taken at the time of training included body weight before and after exercise, temperature, barometric pressure and humidity. From the training data, total revolutions run (TRR) was divided by total expected revolutions (TER, based upon work time, repetitions, run speed and number of bouts) to obtain per cent expected revolutions (PER).

During the first week of training, the PER provided an index for evaluating the conditioned avoidance response (CAR) of each animal. The PER at the conclusion of the program demonstrated the relative ability of an animal to achieve a high level of training compared to his own initial PER and to that of fellow rats within his training group.

Eight to twelve rats were trained at one time in separately numbered running wheels to ensure the same environment. The program was a standard eight-week, medium-duration, moderate-intensity endurance training regimen conducted five days per week. Exact details of the program may be found in the Appendix. Mention should be made, however, of the first and final training day regimens. The initial day of training required three bouts of forty repetitions each involving ten seconds work at 2.0 feet per second and five seconds rest. The schedule of the fortieth day of training included five bouts of eight repetitions requiring thirty seconds work at 4.0 feet per second and thirty seconds rest time. Although total work time remained at 1200 seconds for the first and last day of training, total expected revolutions (TER) increased from 600 on day one to 1200 on day forty. The differences between the program described here and studies conducted in other laboratories in motor driven drums include:

1. the ability of rats to self-propel this apparatus
2. the greater speed at which the animals were trained.

The final speed of 4.0 ft/sec is two to four times the intensity of the training procedures [22, 38] used by other authors in the investigation of the acute effects of running exercise upon catecholamine concentration.

Final Treatment Protocol

Sacrifice procedures were employed two days after the final training day to permit recovery from the acute effects of the training program. This time lag was included to differentiate the acute effects in trained rats from the residual fatigue known to be present in continuous heavy training. However, there are limitations in this concept of residual fatigue. The data on residual fatigue are based on oxygen consumption in man immediately following exercise. There is no certainty that these data are applicable to rats nor that they can be related to CA concentrations in any way. Thus the protocol must be regarded as proceeding from a defined position only, with relatively little evidence to support such a position.

Sedentary and exercised rats were divided into six groups each for a final treatment period as follows:

- E = Trained rat subjected to final exercise program, injected with distilled water 1 1/2 hours prior to session (lasting one hour)
- $E_{a\text{-}MT}$ = Trained rat subjected to final exercise program, injected with alpha-methyltyrosine 1 1/2 hours prior to session
- E_{wh} = Trained rat placed in running wheel secured to prevent rotation for the duration of the session, injected with distilled water 1 1/2 hours prior to placement
- $E_{wh_{a\text{-}MT}}$ = Trained rat placed in running wheel secured to prevent rotation, injected with alpha-methyltyrosine 1 1/2 hours prior to placement

E_{sed} = Trained rat injected with distilled water and immediately placed back in sedentary cage until sacrifice (2 1/2 hours)

$E_{sed_{a\text{-}MT}}$ = Trained rat injected with alpha-methyltyrosine and immediately placed back in sedentary cage until sacrifice (2 1/2 hours)

S_{ch} = Sedentary rat placed in "cheerleader" cage (designed to deliver shock to animal concurrent with shock of trained animal in adjacent running wheel), injected with distilled water 1 1/2 hours prior to session (lasting one hour)

$S_{ch_{a\text{-}MT}}$ = Sedentary rat placed in cheerleader cage, injected with alpha-methyltyrosine 1 1/2 hours prior to session

S_{wh} = Sedentary rat placed in running wheel secured to prevent rotation for the duration of the exercise session, injected with distilled water 1 1/2 hours prior to placement

$S_{wh_{a\text{-}MT}}$ = Sedentary rat placed in running wheel secured to prevent rotation for the duration of the exercise session, injected with alpha-methyltyrosine 1 1/2 hours prior to placement

S_{sed} = Sedentary rat injected with distilled water and immediately placed back in sedentary cage until sacrifice (2 1/2 hours)

$S_{sed_{a\text{-}MT}}$ = Sedentary rat injected with alpha-methyltyrosine and immediately placed back in sedentary cage until sacrifice (2 1/2 hours)

Alpha-methyltyrosine was administered in a dose of 250 mg/kg as the methyl ester hydrochloride,[2] dissolved in

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[2]DL-alpha-methyltyrosine methyl ester hydrochloride was purchased from Regis Chemical Company, Chicago, Illinois under license granted by Merck and Company. This drug represents a more soluble form at physiological pH than the free amino acid.

distilled water and diluted to a 10% solution. This dilution permitted volume injection within 1 cc. Control animals were injected with identical volumes (2.5 mg/kg) of distilled water. Both drug and placebo were administered i.p. 2 1/2 hours prior to sacrifice, or 1 1/2 hours prior to final treatment to those animals undergoing a final treatment session of one hour duration. Thus, the effects of either solution over a 2 1/2 hour period could be evaluated in each animal. This time lag was chosen to allow for significant catecholamine depletion, yet avoid the possibility of total behavioral depression after the administration of this potent tyrosine hydroxylase inhibitor.

At the conclusion of the final treatment session, each rat was removed from the apparatus and sacrificed via decapitation. It was necessary to compensate for the time delay from sacrifice of the first until the last (twelfth) animal in each session. (The elapsed time from the finish of the program until the final decapitation never exceeded ninety seconds.) Therefore, the order of sacrifice of final treatment group at the conclusion of the session was reversed. Following the guillotine procedure, the brain was removed, washed of any excess blood, weighed to the nearest centigram and quick frozen in isopentane chilled by a dry ice, 100% ethanol mixture. Frozen brain tissue was stored in enclosed tin containers at -20°C until analyzed for CA content.

Catecholamine Analysis

The step-by-step procedure presented in Appendix A is based upon the methods used by Moore and Rech [69], which represents a modification of techniques previously outlined for NE [6, 56, 57] and DM [3, 27, 67]. Final CA values of each sample were expressed as ug CA/g of brain. Each analysis was performed without foreknowledge of the specific animal tissue being examined.

Statistical Analysis

Body weight comparisons of exercised and sedentary rats were determined on the basis of weekly difference scores using a correlated "t test." Difference scores between groups were deemed significant if its chance occurrence was .05 or less.

Analysis of CA levels was organized into a replicated two-way analysis of variance (factorial design). One factor represented the final six treatment groups (see Figure 1) and the other compared control (distilled water) to drug (a-MT) treatment. Prior to analysis, all the pertinent observations to be examined were predesigned. This allowed group comparisons to be analyzed with a more powerful statistical tool based upon the technique of Single Degree of Freedom and Least Significant Difference [55]. Utilizing these designed comparisons, per cent depletion (% ug/g/2 1/2 hrs) and differences in depletion (ug/g/2 1/2 hrs) were compared among the various subgroups.

Brain and heart weights were analyzed as total gram weight and per cent body weight using Student's t test.

Finally, a simple correlation matrix was organized for training data, anatomical measurements and CA determinations, incorporating only those variables deemed worthy of comparison.

CHAPTER IV

RESULTS

The presentation of data is based upon the factorial design as presented in the methods section. The decision to use a replicated two-way analysis of variance with factorial design for statistical analysis of the catecholamine data was based upon the need to obtain maximal statistical power for the small sample size utilized in each final treatment subgroup. The probability of chance occurrence was set at the .05 level of significance.

Interpretation of tables and graphs may appear complicated due to the number of groups in the sacrifice protocol and the primary and secondary comparisons which have been made. To aid in this regard, the appropriate group and comparison legends accompany each table or graph. Although the legends appear excessively lengthy, they are necessary to provide the desired independence of each table and graph.

Effectiveness of the Exercise Regimen

The establishment of a favorable behavioral response to the interval training program was of primary concern. A

training effect might be established only if the rats were able to maintain a positive response to the conditioned stimulus, measured as per cent expected revolutions (PER) and total revolutions run (TRR), throughout the forty day program.

The results of two-way analysis of variance on PER and TRR by weeks and by animals from shipment three are shown in Figure 2. There was a steep decline during the sixth week of training ($p < .001$). There is no satisfactory explanation for this observation. The overall PER among those rats chosen for final analysis from each of the shipments did not exceed 65%. However, the average TRR among the trained rats increased from 404 on day two to 648 on the final training day.

Body weight comparisons were made between sedentary and exercised animals as shown in Figures 3 and 4. The gain in body weight was significantly greater among sedentary rats during the first week only. Thereafter, weekly weight gain was similar between both groups.

Brain and heart weights of each group were compared as total gram weight and per cent body weight (Figure 5). Brain and heart weights were significantly higher in the exercise group when expressed as per cent body weight.

The body weight and relative heart weight results indicate that a significant training effect was elicited. The significantly larger relative heart weight in the

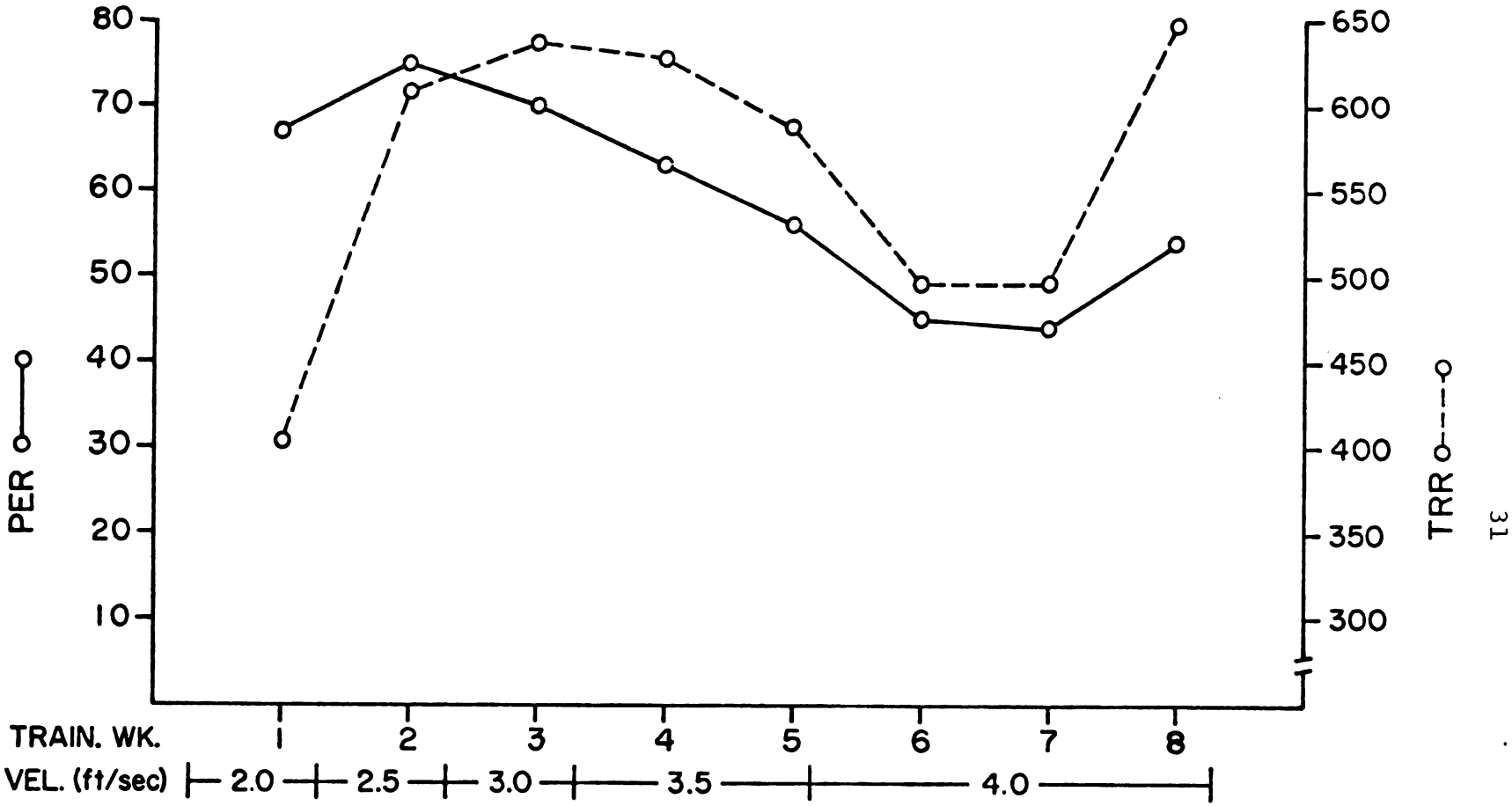

Figure 2: Average percent expected revolutions (PER) and total revolutions run (TRR) of sixteen rats from the first week of training until completion of the program.

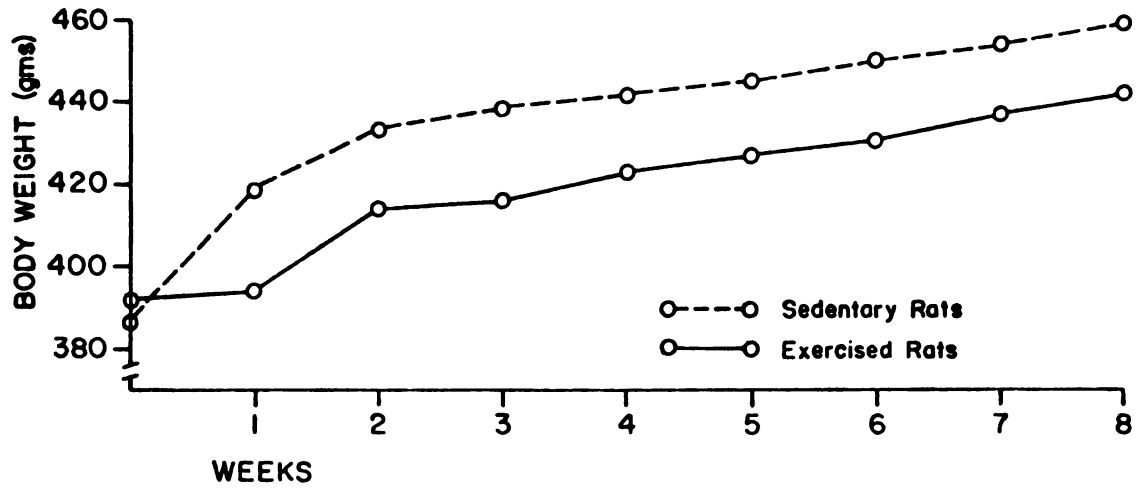

Figure 3: Weekly body weights of sedentary and exercised rats.

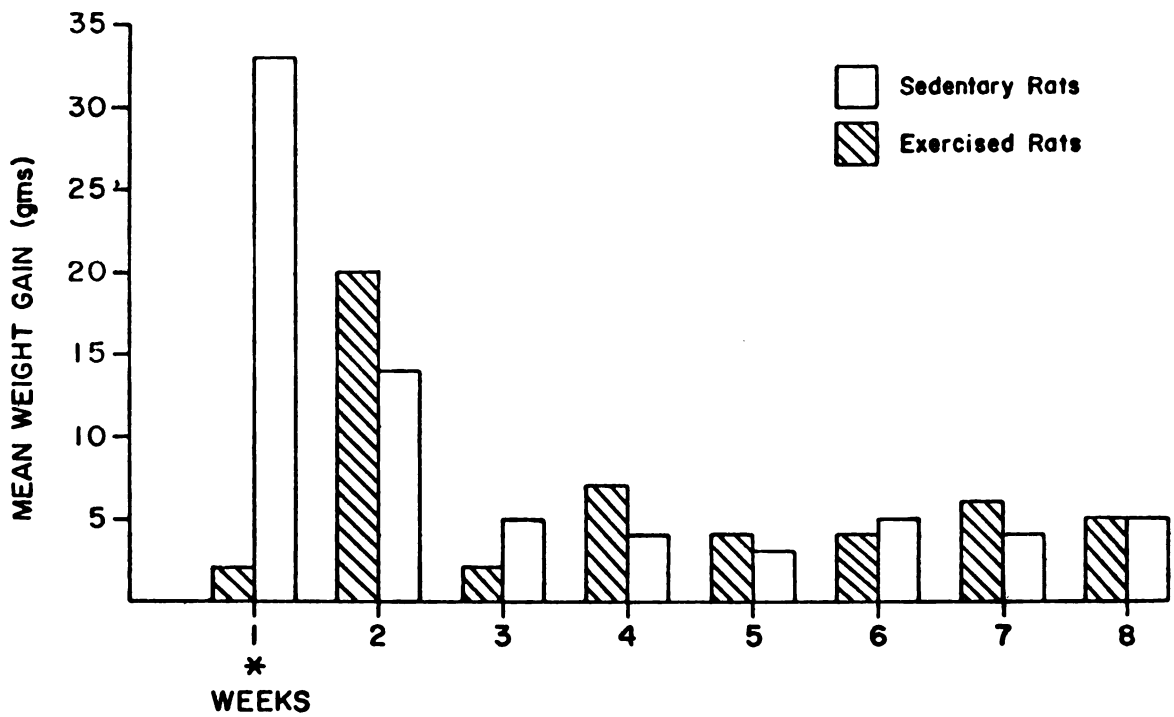

Figure 4: Weekly weight gain of sedentary and exercised rats. Starred comparison is significantly different ($p < .05$).

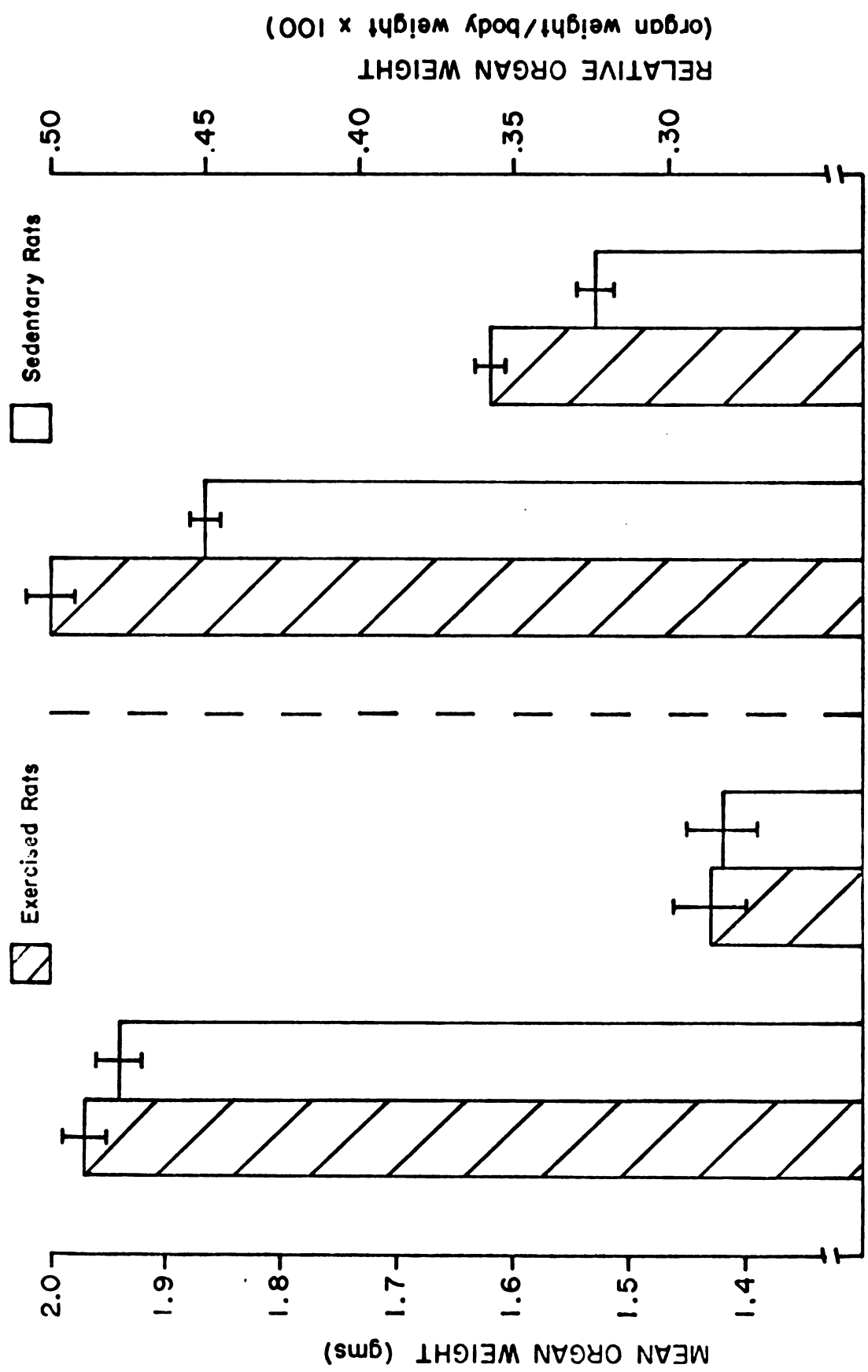

Figure 5: Mean comparison of absolute heart and brain weight expressed as total gram weight and relative heart and brain weight expressed as percent body weight. Vertical lines represent standard error of the mean. Starred comparisons are significant ($p < .05$).

trained animals is a typical training response. The significantly lighter body weight among trained animals also has been observed frequently. However, the fact that the difference in weight of the two groups was initiated in the first week of training only is puzzling. This pattern of group differences is different from previous data utilizing endurance swimming and merits further investigation.

Behavioral Response to Alpha-MT

The ability of a-MT to disrupt CAR and locomotor activity was determined in trained animals subjected to normal exercise routine at final treatment. Cumulative duration of shock (CDS) and TRR were used as criteria to compare exercise rats injected with distilled water (E) to exercise rats injected with a-MT ($E_{a\text{-}MT}$). Table I indicates that a-MT did not statistically affect TRR or CDS among trained rats.

The time course of a-MT administration (1 1/2 hours prior to exercise) was chosen to prevent total sedation of trained animals subjected to the normal exercise routine. This factor coupled with the small sample size may have been instrumental in the lack of a statistically significant difference observed in the behavioral response (TRR and CDS) of physically trained rats to exercise, following injection of distilled water or a-MT (Table I).

TABLE I: Behavioral Response to Alpha-methyltyrosine Among Trained Rats. Numbers represent mean values ± s.e.

Final Treatment	n	Average Cumulative Duration of Shock (seconds)	Average Total Revolutions Run (TRR)
Exercise Control (H_2O)	7	489 ± 62	648 ± 73
Exercise Alpha-MT	7	564 ± 83	626 ± 79
Calculated t value:		.7274	.2033

Catecholamine Response to Alpha-MT

Assessment of the ability of a-MT to lower brain catecholamines was performed on each of the final treatment subgroups. A two-way replicated analysis of variance of brain DM and NE was performed using final treatment subgroups as one variable and the injection media as the other (Table II). The statistical significance of drug treatment observed in Table II demonstrates the ability of a-MT to inhibit synthesis of brain CA stores. The results affirm the purpose for which the drug was employed even though the time course of a-MT activity within each animal was less than that required for maximal synthesis inhibition of brain CA.

TABLE II: Factorial Analysis of Mean Brain Dopamine and Norepinephrine Levels (ug/g) ± s.e. of Sedentary and Exercised Rats Following Final Treatment

	Drug	S_{ch}	S_{wh}	S_{sed}	E	E_{wh}	E_{sed}
DM	Distilled Water	.44 ± .07 n=7	.51 ± .06 n=6	.37 ± .10 n=7	.48 ± .12 n=7	.42 ± .15 n=6	.44 ± .14 n=7
	Alpha-MT	.32 ± .14 n=7	.25 ± .09 n=6	.13 ± .08 n=5	.37 ± .16 n=6	.20 ± .07 n=6	.14 ± .06 n=7
NE	Distilled Water	.31 ± .02 n=7	.37 ± .02 n=6	.34 ± .03 n=7	.38 ± .03 n=7	.42 ± .03 n=6	.45 ± .02 n=7
	Alpha-MT	.22 + .03 n=7	.24 + .04 n=6	.22 + .04 n=6	.26 + .03 n=6	.31 + .04 n=6	.32 + .02 n=7

Two-Way Analysis of Variance (Replicated Design)

Source	Brain DM df	Brain DM MSS	Brain DM F Ratio	Brain DM P: $F_{v1,v2}$	Brain NE df	Brain NE MSS	Brain NE F Ratio	Brain NE P: $F_{v1,v2}$
Final Treatment	5	.0490	.6290		5	.0267	4.3770	p <.005
Drug	1	.3280	4.2105	p <.01	1	.2631	43.1311	p <.005
Interaction	5	.0836	1.0801		5	.0016	.2623	
Error	65	.0774			66	.0061		

Group Legend

Training Condition	Final Treatment
S_{ch} -sedentary	shock
S_{wh} -sedentary	fixed wheel
S_{sed} -sedentary	cage
E -trained	exercise
E_{wh} -trained	fixed wheel
E_{sed} -trained	cage

Selected Group Comparisons of Brain CA

Organizing the data into an orthogonal classification[3] accomplished two purposes. It gave a composite view of the important comparisons in a single table, and secondly, allowed use of a more powerful statistical tool with which to evaluate the data. The comparisons presented in Table III indicate that the average concentration of brain NE among exercised rats was significantly higher than corresponding amine levels of sedentary rats (comparison Q_1). Prior injection of a-MT did not affect this relationship.

The designed comparisons of per cent brain NE and DM depletion over the 2 1/2 hour period subsequent to a-MT injection are graphically presented in Figures 6 and 7, respectively. Ten out of forty-five possible comparisons were chosen for Least Significant Difference analysis in each of the bar graphs.

The results give testimony to the CA depleting ability of a-MT upon brain NE concentrations under all final

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[3]Although used with less frequency than undesigned comparisons, such as Duncan's, Tukey's and Scheffe's tests, the Single Degree of Freedom and Least Significant Difference rely upon selection of the meaningful comparisons before observing any results. The Single Degree of Freedom was used in this study to combine raw data of previously selected subgroups, thereby enlarging the sample size of each comparison and increasing the power of detecting a true difference. Least Significant Difference differs from the above mentioned undesigned analyses by eliminating a correction factor, which adjusts for the inflated Type I error incurred if one wishes to calculate all possible comparisons.

TABLE III: Selected Group Comparisons of Brain CA-Orthogonal Classification.

Catecholamine Variables (ug/g)	Orthogonal Comparisons (Q_i)[a]				"t" Value for each Q_i
	Q_1: $S_{ch}S_{wh}S_{sed}$ vs $EE_{wh}E_{sed}$	Q_2: S_{ch} vs $S_{wh}S_{sed}$	Q_3: E vs $E_{wh}E_{sed}$	Q_4: E_{wh} vs E_{sed}	
Brain NE: H_2O:	.34 .41	.31 .35	.38 .44	.42 .45	
t value:	<u>3.1204</u>[b]	1.1773	1.5616	.6070	1.70
Brain NE: a-MT:	.23 .30	.22 .23	.26 .32	.30 .32	
t value:	<u>2.6371</u>	.2133	1.2432	.2753	1.69
Brain DM: H_2O:	.39 .45	.44 .36	.48 .43	.42 .44	
t value:	.6448	.6274	.3707	.1688	1.69
Brain DM: a-MT:	.24 .28	.32 .20	.37 .17	.20 .14	
t value:	.0748	.9442	1.5053	.3899	1.70

[a]Comparisons (Q_1 through Q_4) represent mean values of final treatment subgroups beneath each horizontal line.

[b]Significant "t" values in the body of the table are underlined.

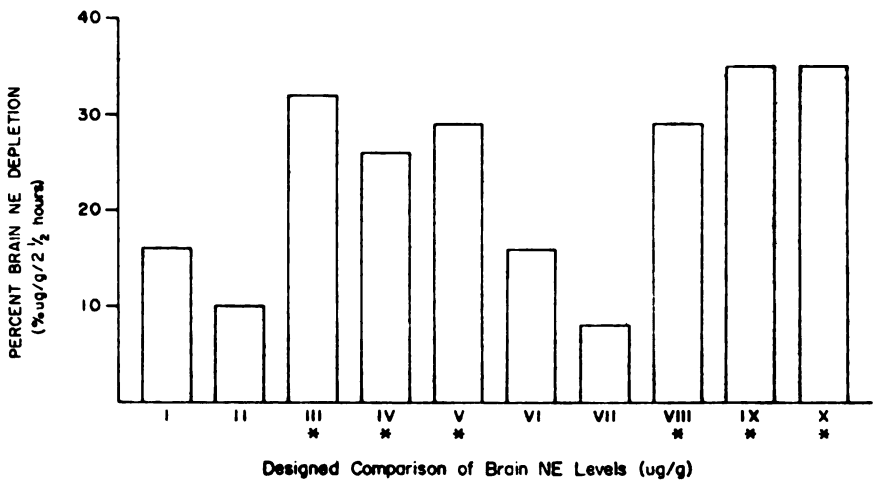

Figure 6: Least significant difference designed comparison of per cent depletion of brain NE levels. Starred comparisons are significant to the .05 level. Mean group values may be found in Table II. Per cent depletion of each comparison (listed in Primary Comparison Legend below) was determined by subtracting the mean group value in column A from the mean group value in column B, dividing the result by the mean group value in column A and multiplying by 100.

$$\left(\frac{B-A}{A} \times 100\right)$$

Group Legend

	Training Condition	Final Treatment	Drug
E	-trained	exercise	H_2O
$E_{a\text{-}MT}$	-trained	exercise	a-MT
E_{wh}	-trained	fixed wheel	H_2O
$E_{wh_{a\text{-}MT}}$	-trained	fixed wheel	a-MT
E_{sed}	-trained	cage	H_2O
$E_{sed_{a\text{-}MT}}$	-trained	cage	a-MT
S_{ch}	-sedentary	shock	H_2O
$S_{ch_{a\text{-}MT}}$	-sedentary	shock	a-MT
S_{wh}	-sedentary	fixed wheel	H_2O
$S_{wh_{a\text{-}MT}}$	-sedentary	fixed wheel	a-MT
S_{sed}	-sedentary	cage	H_2O
$S_{sed_{a\text{-}MT}}$	-sedentary	cage	a-MT

Primary Comparison Legend

	A		B	Effects of:
I	$E_{a\text{-}MT}$	vs	$E_{wh_{a\text{-}MT}}$	:exercise and/or shock upon trained rats without CA synthesis
II	E	vs	E_{wh}	:exercise and/or shock upon trained rats with CA synthesis
III	$E_{a\text{-}MT}$	vs	E	:a-MT upon trained rats after acute exercise
IV	$E_{wh_{a\text{-}MT}}$	vs	E_{wh}	:a-MT upon trained rats without exercise in fixed wheel
V	$E_{sed_{a\text{-}MT}}$	vs	E_{sed}	:a-MT upon trained rats without exercise in cages
VI	S_{ch}	vs	S_{wh}	:shock upon sedentary rats with CA synthesis
VII	$S_{ch_{a\text{-}MT}}$	vs	$S_{wh_{a\text{-}MT}}$	:shock upon sedentary rats without CA synthesis
VIII	$S_{ch_{a\text{-}MT}}$	vs	S_{ch}	:a-MT upon sedentary rats after shock
IX	$S_{wh_{a\text{-}MT}}$	vs	S_{wh}	:a-MT upon sedentary rats in fixed wheel
X	$S_{sed_{a\text{-}MT}}$	vs	S_{sed}	:a-MT upon sedentary rats without exercise in cages

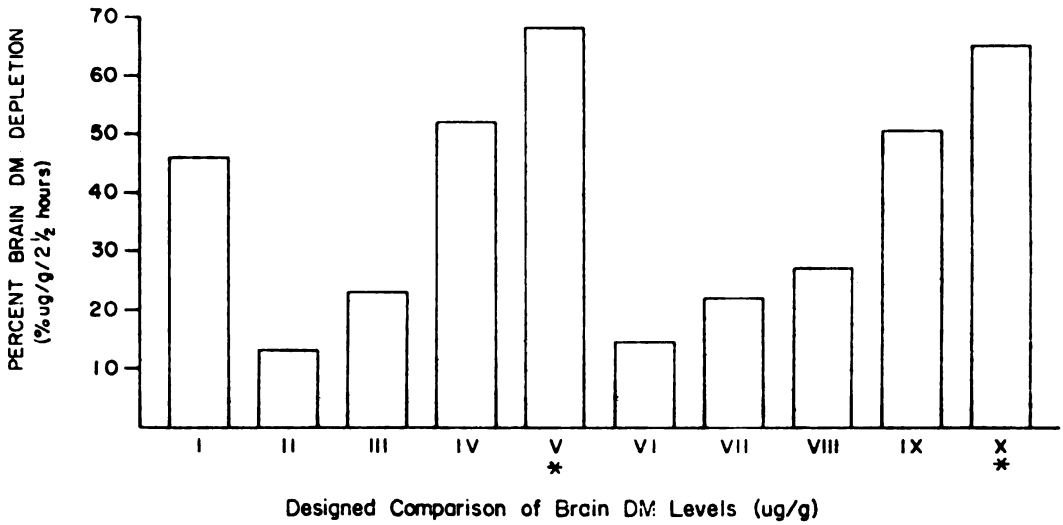

Figure 7: Least significant difference designed comparison of per cent depletion of brain DM levels. Starred comparisons are significant to the .05 level. Mean Group values may be found in Table II. Per cent depletion of each comparison (listed in Primary Comparison Legend below) was determined by subtracting the mean group value in column A from the mean group value in column B, dividing the result by the mean group value in column B and multiplying by 100.

$(\frac{B-A}{A} \times 100)$

Group Legend

	Training Condition	Final Treatment	Drug
E	-trained	exercise	H_2O
$E_{a\text{-}MT}$	-trained	exercise	a-MT
E_{wh}	-trained	fixed wheel	H_2O
$E_{wh_{a\text{-}MT}}$	-trained	fixed wheel	a-MT
E_{sed}	-trained	cage	H_2O
$E_{sed_{a\text{-}MT}}$	-trained	cage	a-MT
S_{ch}	-sedentary	shock	H_2O
$S_{ch_{a\text{-}MT}}$	-sedentary	shock	a-MT
S_{wh}	-sedentary	fixed wheel	H_2O
$S_{wh_{a\text{-}MT}}$	-sedentary	fixed wheel	a-MT
S_{sed}	-sedentary	cage	H_2O
$S_{sed_{a\text{-}MT}}$	-sedentary	cage	a-MT

Primary Comparison Legend

	A		B	Effects of:
I	$E_{wh_{a\text{-}MT}}$	vs	$E_{a\text{-}MT}$	:exercise and/or shock upon trained rats without CA synthesis
II	E_{wh}	vs	E	:exercise and/or shock upon trained rats with CA synthesis
III	$E_{a\text{-}MT}$	vs	E	:a-MT upon trained rats after acute exercise
IV	$E_{wh_{a\text{-}MT}}$	vs	E_{wh}	:a-MT upon trained rats without exercise in fixed wheel
V	$E_{sed_{a\text{-}MT}}$	vs	E_{sed}	:a-MT upon trained rats without exercise in cages
VI	S_{ch}	vs	S_{wh}	:shock upon sedentary rats with CA synthesis
VII	$S_{wh_{a\text{-}MT}}$	vs	$S_{ch_{a\text{-}MT}}$	:shock upon sedentary rats without CA synthesis
VIII	$S_{ch_{a\text{-}MT}}$	vs	S_{ch}	:a-MT upon sedentary rats after shock
IX	$S_{wh_{a\text{-}MT}}$	vs	S_{wh}	:a-MT upon sedentary rats in fixed wheel
X	$S_{sed_{a\text{-}MT}}$	vs	S_{sed}	:a-MT upon sedentary rats without exercise in cages

treatment conditions and for both experiment groups (comparisons III, IV, V, VIII, IX and X).

Brain DM concentrations experienced significant decline among a-MT treated trained and sedentary rats under sedentary housing conditions (Figure 7, comparisons V and X). A-MT did not significantly lower brain DM among trained rats subjected to exercise (III), placed in a fixed running wheel (IV), or among sedentary rats placed in "cheerleader" cages (VIII) and secured running wheels (IX).

Depletion Comparisons Between Final Treatment Subgroups

Least Significant Difference was used, in addition, to determine the effect of differences in depletion among the various subgroups listed in Table IV.

The results indicate exercise and/or shock potentiates a-MT-induced depletion of brain NE among trained rats (I vs III). DM depletion was not augmented under the same circumstances. Table IV also demonstrates increased a-MT-induced depletion of brain NE among trained rats subjected to exercise compared to shock depleted NE values among sedentary rats (III vs VII). Therefore, exercise and/or shock among trained rats may have evoked greater utilization of brain NE stores than did shock among sedentary animals. No significant effects were observed for brain DM comparisons.

TABLE IV: Depletion comparisons between final treatment subgroups.

Subgroups $\bar{i} - \bar{j}$	Mean differences ($\bar{i} - \bar{j}$, ug/g/2 1/2 hours) Brain NE	Brain DM
I - VII	-.03	-.10
IV - IX	.02	.04
VI - VII	-.04	-.14
IV - V	.02	.08
IX - X	-.01	-.02
III - IV	-.01	.11
I - III	.07**	-.06
III - VII	-.10**	-.04
VIII - IX	.04	-.14

** $p < .05$.

Group Legend

	Training Condition	Final Treatment	Drug
E	-trained	exercise	H_2O
$E_{a\text{-}MT}$	-trained	exercise	a-MT
E_{wh}	-trained	fixed wheel	H_2O
$E_{wh_{a\text{-}MT}}$	-trained	fixed wheel	a-MT
E_{sed}	-trained	cage	H_2O
$E_{sed_{a\text{-}MT}}$	-trained	cage	a-MT
S_{ch}	-sedentary	shock	H_2O
$S_{ch_{a\text{-}MT}}$	-sedentary	shock	a-MT
S_{wh}	-sedentary	fixed wheel	H_2O
$S_{wh_{a\text{-}MT}}$	-sedentary	fixed wheel	a-MT
S_{sed}	-sedentary	cage	H_2O
$S_{sed_{a\text{-}MT}}$	-sedentary	cage	a-MT

Primary Comparison Legend

	A		B	Effects of:
I	$E_{a\text{-}MT}$	vs	$E_{wh_{a\text{-}MT}}$	:exercise and/or shock upon trained rats without CA synthesis
II	E	vs	E_{wh}	:exercise and/or shock upon trained rats with CA synthesis
III	$E_{a\text{-}MT}$	vs	E	:a-MT upon trained rats after acute exercise
IV	$E_{wh_{a\text{-}MT}}$	vs	E_{wh}	:a-MT upon trained rats without exercise in fixed wheel
V	$E_{sed_{a\text{-}MT}}$	vs	E_{sed}	:a-MT upon trained rats without exercise in cages
VI	S_{ch}	vs	S_{wh}	:shock upon sedentary rats with CA synthesis
VII	$S_{ch_{a\text{-}MT}}$	vs	$S_{wh_{a\text{-}MT}}$	:shock upon sedentary rats without CA synthesis
VIII	$S_{ch_{a\text{-}MT}}$	vs	S_{ch}	:a-MT upon sedentary rats after shock
IX	$S_{wh_{a\text{-}MT}}$	vs	S_{wh}	:a-MT upon sedentary rats in fixed wheel
X	$S_{sed_{a\text{-}MT}}$	vs	S_{sed}	:a-MT upon sedentary rats without exercise in cages

Secondary Comparison Legend

Comparison	Meaning
I vs VII	:Does a trained rat subjected to exercise and/or shock deplete his CA stores (without synthesis taking place) to a greater degree than a sedentary rat subjected to shock?
IV vs IX	:Does the anxiety of placing a trained rat in the running wheel who expects to run but cannot do so, potentiate a-MT induced depletion of CA compared to a sedentary animal under similar housing conditions?
VI vs VII	:Does the ability to synthesize CA under shock stress decrease the depletion of CA stores in the sedentary rat, or does behavioral depression (due to a-MT) prevent an anxiety (shock) response in sedentary rats from decreasing its depletion of CA?
IV vs V	:Does the anxiety of placing a trained rat in the running wheel who expects to run but cannot do so, potentiate a-MT induced depletion of CA stores compared to trained rats in sedentary cages?
IX vs X	:Will sedentary rats respond to a new environment by increasing a-MT induced depletion of CA?
III vs IV	:Does acute exercise and/or shock among trained rats potentiate a-MT induced CA depletion compared to the trained rat placed in a similar environment but prevented from performing his normal (expected) pattern of response?
I vs III	:Does exercise and/or shock potentiate a-MT induced CA depletion among trained rats?
III vs VII	:Comparison of a-MT induced CA depletion after acute exercise among trained rats to shock induced CA depletion among sedentary rats.
VIII vs IX	:Does shock potentiate a-MT induced CA depletion in sedentary rats?

Behavioral Response and Brain CA Concentration

The correlational analysis between brain CA and motor activity presented in Table V may assist in differentiating the relative contribution of DM and NE during exercise. A moderately negative correlation (r = -.62) between brain DM and TRR during final treatment among trained rats subjected to exercise and a-MT may indicate that this amine was utilized at a faster rate than it was synthesized.

TABLE V: Final Treatment Correlations: Behavioral Response and Catecholamine Concentration in the Brain.

Catecholamine	Final Group	n	TRR	CDS
Brain NE (ug/g)	E[1]	7	.33	-.32
	$E_{a\text{-}MT}$	6	-.06	.13
Brain DM (ug/g)	E	7	-.28	.27
	$E_{a\text{-}MT}$	6	-.62	.64

[1]E--trained, exercise, H_2O; $E_{a\text{-}MT}$--trained, exercise, a-MT.

Relationship Between Brain CA and Brain Weight

Low correlations were observed between brain CA and brain weight (Table VI). Apparently, the size of the brain does not dictate the concentration of DM or NE contained within.

TABLE VI: Relationship Between Brain CA and Brain Weight.

Comparison	Trained	n	Sedentary	n
Brain Weight vs Brain NE (grams) (ug)	.34	39	.39	38
Brain Weight vs Brain DM (grams) (ug)	.08	39	.17	37

Discussion

An evaluation of the training data and body and organ weight comparisons between exercise and sedentary animals shows that the regimen employed did produce a significant training effect. However, too many unanswered questions remain which qualify any conclusions regarding the training program. The accumulation of shock time in the final days of the program may indicate that the exercise group adapted to shock stress. Until this training program is clarified further, the results obtained in this study can be applied only to adult, male, albino rats subjected to a similar training regimen.

The implications that can be drawn from the CA analysis of brain tissue is speculative at best. The statistically significant results of brain CA comparisons require cautious interpretation until a more specific role is assigned to NE and DM in the functioning of the central nervous system. If one assumes NE modulates the central component of sympathetic tone, then the increased brain NE

levels observed among trained rats (compared to sedentary controls) may be indicative of a higher maintained level of sympathetic output.

The ability of a-MT to deplete brain NE under all the conditions imposed in the final treatment gives testimony to its function as a powerful synthesis inhibitor, most likely of tyrosine hydroxylase [93]. However, the presence of control brain DM values among trained rats subjected to exercise and a-MT suggests conservation of this amine.

Exercise and/or shock among trained and sedentary rats did not significantly lower brain NE or DM concentrations (Figures 6 and 7, comparison I). Gordon et al. [38] observed the same result following one hour of treadmill exercise of previously sedentary rats. However, significant depletion of rat brain NE following electric shock (5 ma) through a grid floor for one hour [9, 60] was not corroborated in the present investigation. This may have resulted from the lower shock stimulus (1.2 ma) used by this author. Thierry et al. [95] obtained results similar to those of the current investigation in rats following intermittent grid shock (0.8 ma) for three hours.

Least Significant Difference analysis demonstrated the ability of exercise and/or shock to augment brain NE depletion in trained rats (Table IV, comparison I vs III). This is comparable to the data of Gordon et al. [38] indicating an increased turnover of brain NE following one hour

of running exercise. These authors obtained one-half the depletion following a-MT injection in, roughly, one-half the time period used in this study.

Exercise did not increase the rate of brain DM depletion in trained rats treated with a-MT. This implies the presence of a mechanism designed to conserve DM under conditions of extreme stress (drug plus exercise). If brain DM were responsible for the maintenance of motor activity as suggested by many authors [7, 15, 75, 86] it would appear that such a mechanism is operating.

Stores of brain NE were depleted to a greater extent in trained rats exposed to exercise compared to sedentary rats subjected to shock the final day (Table IV, III vs VII). The exercise routine appears to have evoked a greater sympathetic discharge than shock stress alone.

In conclusion, a model is proposed incorporating the theory of Brodie and others [11] placing emphasis upon brain DM stores in the regulation of motor activity under conditions of extreme stress acting somewhat independently of sympathetic stimulation during exercise, modulated by brain NE.

CHAPTER V

SUMMARY, CONCLUSIONS, RECOMMENDATIONS

It was the purpose of this investigation to demonstrate the chronic effects of a specific exercise regimen upon rat brain catecholamine concentrations.

Forty male albino rats were exercised in an electronically controlled running wheel five days per week for a total of eight weeks. An equal number of animals served as sedentary controls. Daily records of animal weight and performance were kept during the training period.

The animals were divided into six subgroups during the final treatment protocol. Half of each group was injected i.p. with distilled water, and the remainder with alpha-methyltyrosine, a potent catecholamine synthesis inhibitor [93]. One and one-half hours following injection, each rat was exposed to his final treatment as follows:

E = Trained rat run through "normal" exercise routine

E_{wh} = Trained rat placed in running wheel secured to prevent rotation

E_{sed} = Trained rat placed in sedentary cage

S_{ch} = Sedentary rat placed in "cheerleader" cage (subjecting animal to the identical amount of shock as E)

S_{wh} = Sedentary rat placed in running wheel secured to prevent rotation

S_{sed} = Sedentary rat placed in sedentary cage.

All animals were decapitated one hour following final treatment. Brain and heart were removed, weighed, quick-frozen and the brain subsequently analyzed for catecholamine content, fluorometrically.

Tissue catecholamine content was statistically analyzed using a replicated two-way analysis of variance (factorial design). Variables were further compared using the Single Degree of Freedom and Least Significant Difference designed comparisons. These techniques were employed to gain power in detecting true differences between subgroups and various combinations of subgroups.

On the basis of prior studies investigating the acute effects of a variety of stress situations, it was the author's belief that brain catecholamine levels might be altered in a differential fashion under the experimental conditions imposed.

Conclusions

Conclusions to be drawn from this study are as follows:

1. Increased steady-state levels of brain norepinephrine observed in physically trained rats may have been caused by the continuous demand placed upon the sympathetic

nervous system to which this amine has been linked as neurotransmitter.

2. The inability of alpha-methyltyrosine to deplete the brain of dopamine among trained rats exposed to stress may implicate this amine in the control of motor function and may suggest a mechanism which tends to bypass the effects of synthesis inhibition in situations requiring excessive amounts of dopamine.

Recommendations

1. It is suggested that training procedures be limited to a maximum of six rats per session (in lieu of twelve per session) continuously modifying the regimen according to the response of each animal.

2. Techniques need to be altered to allow investigation of resting heart rate and systolic blood pressure, and their relationship to heart and brain CA concentrations.

3. A time study with larger sample sizes per group is recommended to accurately evaluate turnover rates of brain catecholamines.

4. The electronically controlled running wheel used in this study to bring about training effects due to exercise must be subjected to careful scrutiny to prevent chronic adaptation to shock stress.

5. Further clarification of the internal metabolic changes is needed to explain the reduction in the body weight of rats during the first week of the training regimen.

It is the author's opinion that greater research effort be applied to clarify the central neurochemical changes taking place after chronic exposure to different exercise regimens.

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APPENDIX

APPENDIX A

BIOCHEMICAL ANALYSIS OF CATECHOLAMINES

Heart and brain tissue was permitted to thaw prior to homogenization in 6 ml of cold 0.4 N perchloric acid. The toughness of the heart tissue required cold-controlled grinding in a ten broeck pyrex tissue grinder, however, the softer brain substance was quickly ground in a teflon pestle tissue grinder. The homogenates were maintained in an ice bath for 30 minutes and then centrifuged for five minutes at 10,000 x g. The supernatant was obtained, and the procedure repeated, combining final eluates into a single sample adjusted to pH 4.0 with 10 N KOH and 1 N KOH. Centrifugation separated the potassium perchlorate precipitate from the supernatant which was then added to 50 ml glass-stoppered centrifuge tubes containing about 400 mg of prepared aluminum oxide (Woelm) and 0.5 ml of 0.2 M disodium ethylenediaminetetraacetate. DM (2.0 ug) and NE (0.8 ug) were used as standards and run through the alumina exchange along with samples. The pH of the combined alumina-tissue mixture was adjusted to 8.6 to 8.7 with 5M K_2CO_3 and 0.2 M K_2CO_3. The supernatant was removed by aspiration, washed

twice with 10 ml H_2O and shaken for five minutes subsequent to each washing. The amines were eluted with 8.0 ml of 0.2 N acetic acid prior to the final shake (of 10 minutes duration) and centrifuged for 10 minutes. The final eluate was divided into two 4 ml aliquots and transferred to "DM" or "NE" assay tubes for subsequent analysis. No more than eight tubes were shaken concurrently.

The pH of the 4 ml "NE" eluate was brought to 6.5 to 6.8 with 5 M K_2CO_3 followed by the addition of 0.8 ml of pH 6.5 phosphate buffer to each tube. Each eluate was then divided into two 2.4 ml aliquots, one serving as blank, the other for NE determination.

NE Fluorescent Procedure

Samples: (Tubes shaken thoroughly after each addition)

1. Start timer and add .05 ml of 0.25% potassium ferricyanide.
2. After 2 minutes add freshly prepared alkaline ascorbate (20 mg ascorbic acid plus 1.0 ml H_2O plus 9.0 ml 5 N NaOH).

Blanks:

1. Add 0.25 ml of alkaline ascorbate.

Fluorescence was determined within 10 minutes in an Aminco-Bowman spectrophotofluorometer at activation-fluorescent wavelengths of 391 and 510 mu respectively.

Mean recovery of heart NE was 70% ± 3.4 (S.D.), with a slightly higher average for brain (77% ± 7.1).

DM was determined adjusting the 4.0 ml acid eluate to pH 6.2 to 6.5 by addition of 2.0 ml of phosphate buffer (pH 8.0). 3.0 ml of the above mixture was removed for assay, the remaining 3.0 ml serving as a blank.

DM Fluorescent Procedure

Sample:

1. Add 0.2 ml of 0.5% sodium periodate (mix).
2. Wait 1 minute exactly.
3. Add 1.0 ml of alkaline sulfite (2.65 g of Na_2SO_3 + 10 ml H_2O + 90 ml of 5 N Na).
4. In rapid succession add:
   a. 2.8 ml H_2O
   b. 1.0 ml of 0.5 M Citrate buffer
   c. 1.7 ml of 3 M phosphoric acid.

Fluorescence was determined ten minutes later in an Aminco-Bowman spectrophotofluorometer at activating-fluorescent wavelengths of 325 and 385 mu, respectively. Recovery of brain DM was 65% ± 14.1.

Blanks were subtracted from sample fluorescence (at meter multiplier setting .03), divided by the fluorescent value of the standards run through with each analysis and multiplied by the content of CA within the standard cuvettes (0.2 ug for NE and 0.5 ug for DM) to obtain concentrations.

TABLE A-1: Brain Catecholamine Correlations.

Final Treatment Groups	n	Brain NE vs Brain DM (ug/g) (ug/g)
E	7	.48
$E_{a\text{-}MT}$	6	.66
E_{wh}	6	.28
$E_{wh_{a\text{-}MT}}$	6	.67
E_{sed}	7	.62
$E_{sed_{a\text{-}MT}}$	7	.59
S_{ch}	7	.66
$S_{ch_{a\text{-}MT}}$	7	.68
S_{wh}	6	.77
$S_{wh_{a\text{-}MT}}$	6	.28
S_{sed}	7	.42
$S_{sed_{a\text{-}MT}}$	5	.83

TABLE A-2.--Standard eight-week, medium-duration, moderate-intensity endurance training program for postpubertal and adult male rats in controlled-running wheels.

Week	Day of Week	Day of Train.	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
1	1=M	1	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	2=T	2	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	3=W	3	3.0	00:10	10	30	4	2.5	1.2	2.0	39:45	600	1200
	4=T	4	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
	5=F	5	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
2	1=M	6	2.0	00:10	10	28	4	5.0	1.0	2.5	51:40	700	1120
	2=T	7	1.5	00:10	10	27	4	5.0	1.0	3.0	50:20	810	1080
	3=W	8	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	4=T	9	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	5=F	10	1.5	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
3	1=M	11	1.0	00:10	10	27	4	5.0	1.2	3.0	50:20	810	1080
	2=T	12	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	3=W	13	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	4=T	14	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	5=F	15	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
4	1=M	16	1.5	00:10	10	26	4	5.0	1.0	3.5	49:00	910	1040
	2=T	17	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	3=W	18	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	4=T	19	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	5=F	20	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
5	1=M	21	1.5	00:15	15	19	4	5.0	1.0	3.5	52:00	997	1140
	2=T	22	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	3=W	23	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	4=T	24	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	5=F	25	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
6	1=M	26	1.5	00:15	15	14	5	5.0	1.0	4.0	53:45	1050	1050
	2=T	27	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	3=W	28	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	4=T	29	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	5=F	30	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
7	1=M	31	1.5	00:20	20	11	5	5.0	0.8	4.0	55:00	1100	1100
	2=T	32	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	3=W	33	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	4=T	34	1.5	00.25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	5=F	35	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
8	1=M	36	1.5	00:25	25	9	5	5.0	0.8	4.0	55:25	1125	1125
	2=T	37	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	3=W	38	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	4=T	39	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200
	5=F	40	1.5	00:30	30	8	5	5.0	0.8	4.0	57:30	1200	1200

This standard program was designed using male rats of the Sprague-Dawley strain. All animals were between 70 and 170 days-of-age at the beginning of the program. The duration and intensity of the program were established so that 75 per cent of all such animals should have PSF and PER scores of 75 or higher during the final two weeks. Alterations in the work time, rest time, repetitions per bout, number of bouts, or time between bouts can be used to affect changes in these values. Other strains or ages of animals could be expected to respond differently to the program.

All animals should be exposed to a minimum of one week of voluntary running in a wheel prior to the start of the program. Failure to provide this adjustment period will impose a double learning situation on the animals and will seriously impair the effectiveness of the training program.

Standard medium-duration, moderate-intensity endurance maintenance program for postpubertal and adult male rats in controlled-running wheels.

	Acceleration Time (sec)	Work Time (min:sec)	Rest Time (sec)	Repetitions per Bout	Number of Bouts	Time Between Bouts (min)	Shock (ma)	Run Speed (ft/sec)	Total Time of Prog. (min:sec)	Total Exp. Revolutions TER	Total Work Time (sec) TWT
	1.5	00:30	30	8	3	5.0	0.8	4.0	32:30	720	720