Squire, Jonathan M.

VOLUNTARY EXERCISE, CHRONIC STRESS, AND BLOOD PRESSURE AND HEART RATE RESPONSES IN THE BORDERLINE HYPERTENSIVE RAT

City University of New York

PH.D. 1986

PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark __✓__.

1. Glossy photographs or pages _____
2. Colored illustrations, paper or print _____
3. Photographs with dark background _____
4. Illustrations are poor copy _____
5. Pages with black marks, not original copy _____
6. Print shows through as there is text on both sides of page _____
7. Indistinct, broken or small print on several pages ✓
8. Print exceeds margin requirements _____
9. Tightly bound copy with print lost in spine _____
10. Computer printout pages with indistinct print _____
11. Page(s) _____ lacking when material received, and not available from school or author.
12. Page(s) _____ seem to be missing in numbering only as text follows.
13. Two pages numbered _____. Text follows.
14. Curling and wrinkled pages _____
15. Dissertation contains pages with print at a slant, filmed as received ✓
16. Other _____

_____

_____

University
Microfilms
International

VOLUNTARY EXERCISE, CHRONIC STRESS, AND BLOOD PRESSURE AND HEART RATE RESPONSES IN THE BORDERLINE HYPERTENSIVE RAT

by

Jonathan M. Squire

A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1986

This manuscript has been read and accepted for the Graduate Faculty in Psychology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

12.13.1985 — Date

Robert Fried — Dr. Robert Fried, Chairman of Examining Committee

12/16/85 — Date

Herbert D. Saltzstein — Dr. Herbert Saltzstein, Executive Officer

Dr. Gordon Barr

Dr. Nori Geary

Dr. Michael Myers

Dr. Gerald Turkewitz

Supervisory Committee

The City University of New York

Abstract

Voluntary Exercise, Chronic Stress, and Blood Pressure and Heart Rate Responses in the Borderline Hypertensive Rat

by

Jonathan M. Squire

Adviser: Dr. Robert Fried

The present investigation was concerned with the effect of voluntary exercise in attenuating increases in blood pressure (BP) and heart rate (HR) caused by various stressors in an animal model of borderline hypertension, the borderline hypertensive rat (BHR). Chronic stress has been shown to increase both BP and HR in humans and animals. Forced exercise is known to lower HR, but has equivocal effects on BP. In the first experiment, adult BHR's were subjected to one week of signalled shock followed by six weeks of signalled, unsignalled, or no shock (handled controls). Half of these animals also had free access to a running wheel. BP and HR were measured before and after these treatments. Exercise was found to attenuate the rise in BP and HR due to stress. The exercise effect was most pronounced in the no shock group. These results suggested that voluntary exercise can attenuate increases in HR due to stress, and may be most effective in combination with certain types of stressors. In a pilot experiment, BP was measured via an indwelling catheter. BP increased upon transfer to the shock chamber and upon shock delivery. BP decreased to baseline levels immediately after removal from the shock chamber. In the second experiment, BHR's were subjected daily to anticipation of shock or handling for six weeks. Half of these animals could exercise voluntarily. Control groups for the effects of social isolation were

also included. BP and HR were measured weekly under ether anesthesia. BP and HR increased due to both stresses. BP and HR also tended to increase in the socially isolated control groups, possibly due to repeated ether stress. The effect of freely available exercise was found to attenuate stress-induced increases in HR, but not BP. Voluntary exercise may have altered autonomic nervous system responsiveness, local hemodynamic processes in skeletal muscle, and/or resting hormone levels. In the third study, social isolation for one week increased HR. These studies demonstrated that in this animal model, voluntary exercise can reliably attenuate increases in HR but not BP due to chronic stressors and can be an effective protocol for exercise training in rats, and that relatively mild stressors can demonstrably affect BP and HR.

Acknowledgements

Dr. Robert Fried, my dissertation advisor, has been an invaluable source of support in the completion of my graduate school tenure. His encouragement and enthusiasm has been instrumental in my quest for the doctorate in psychology.

I have been very fortunate to have done much of my disseration research in the laboratory of Dr. Michael Myers. He has always been willing to listen, support, and assist me in the collection and evaluation of this research, and in the many "little details" necessary for such a project.

My other dissertation committee members, Drs. Gordon Barr, Nori Geary, and Gerald Turkewitz deserve a round of applause for their careful reading and critical and thoughtful comments and suggestions.

Shidan Tavana was also very helpful for technical assistance in the designing of the required equipment and software. Harry Shair, I thank you for your friendship, patience, helpful suggestions, and in knowing where to find everything in the lab. I am also grateful to Lily Skaredoff for her infinite patience during surgical procedures.

Finally, I would like to thank my family, particularily my sister Susan and father David Squire, who were always willing to listen and advise me every step of the way, and whose unqualified support was and remains very important to my development.

Jonathan M. Squire

December, 1985

Table of Contents

List of Tables

List of Figures

INTRODUCTION

Risks Posed by High Blood Pressure and Heart Rate

Human essential hypertension, a major risk factor for coronary heart disease (Rosenman, Friedman, Straus, Wurm, Kositch, Hahn, and Werthessen, 1964), is characterized by high blood pressure associated with an elevated overall resistance to blood flow in peripheral vessels (TPR: Weiner, 1979). Numerous researchers have suggested that repeated psychologically stressful stimuli, when combined with some genetic predisposition, may trigger sustained hypertension (HT) (Lawler, Barker, Hubbard, & Allen, 1980; Folkow, 1978). For instance, certain stressful events such as rapid cultural change, migration, and socioeconomic mobility have been shown to be associated with elevated blood pressure (Guttman & Benson, 1971).

Tachycardia (TC), an abnormally increased heart rate (HR), has also been associated with chronic stress (Paul, Lepper, Ostfeld, MacMillan, & Phelan, 1962). When prolonged, TC can also compromise health. Chronic TC, and its associated severe left ventricular hypertrophy, will increase the risk of myocardial failure (Coleman, Taylor, Pool, Whipple, Covell, Ross, & Braunwald, 1971).

The hypertensive and tachycardic effects of stressful situations in individuals at risk for HT, such as having one hypertensive parent, have been found to be greater than in a nonhypertensive population (Falkner, Onesti, Angelako, Fernandes, & Langman, 1979; Manuck, Proietti, Rader, & Polefrone, 1985). These borderline hypertensive individuals may be

particularly susceptible to developing essential HT and/or tachycardia in the presence of chronic environmental stress, as compared to individuals with no such genetic predisposition (Manuck, Giordani, McQuaid, & Garrity, 1981).

The Concept of Stress.

Since the present research is concerned with the effects of stress, a brief discussion of the concept and definition of stress is now presented. Stress is thought to be a response of the organism to external or internal events known as "stressors". The definition of stress has been controversial from its inception. Walter B. Cannon described adjustments of the organism to potentially stressful events as "homeostatic", i.e., attempts to maintain the stability of the internal environment (Cannon, 1932). He noted that similar adjustments were made in response to different kinds of potentially stressful environmental events (Cannon, 1915).

The immunologist, Hans Selye, was the first to catalogue the stress response (Selye, 1936). In The Stress of Life (1976, p.1), Selye defined stress as "the nonspecific response of the body to any demand". However, the "nonspecific nature" of the stress response has been challenged (e.g., Lenox, Kant, Sessions, Pennington, Mougey, & Meyeroff, 1980; Mason, Maher, Hartley, Mougey, Perlow, & Jones, 1976; Cohen & Obrist, 1975). Both the qualitative and quantitative adjustments made by the body are subtly dependent on type, intensity, and perceptual evaluation of the potential stressors. The physiological adjustments made during mild exercise, for instance, are quantitatively different from those during strenuous exercise (Robinson, Epstein,

or nociceptive stimulation (Cohen & Obrist, 1975).

The reaction to a potential stressor also differs with the way the organism perceives it. For example, perception of control over the stimuli results in a lessor stress response than perception of lack of control (Miller, 1980). This distinction is particularly relevant in the present report, where the imposition of exercise may give different results than the voluntary instigation of a similar activity.

Attempts to identify sensitive and reliable visceral indices of stress have been more successful than defining the stress response based on external circumstances. The most promising of these visceral indices have been plasma levels of the catecholamines (CA) - epinepherine (E) and norepinepherine (NE) - and the corticosteroids, which include cortisol in man, and corticosterone (C) in other mammals. Although C is a reliable indicator of the emotional activation associated with stress (Bush, 1962), its sensitivity has been questioned (Natelson, Tapp, Adamus, Mittler, & Levin, 1981). Levels of corticosteroids often peak at low levels of stress and show no further increase with increasing stressor intensity, in rats (Friedman, Ader, Grota, & Larson, 1967), and in monkeys (Natelson, Krasnagor, & Holaday, 1976). In the review of Natelson et al. (1981), it was concluded that plasma CA levels were the best visceral index at present of the stress response, since these levels reflect both the presence and intensity of the stimulation.

The Definition of Stress.

Stress is a coordinated and multifaceted response, characterized by phasic increases in plasma levels of catecholamines and corticosteroids, among other things. A stressor, then, may be defined as any external stimulus which produces these effects. According to this definition, a

stimulus which produces these effects. According to this definition, a program of voluntary exercise would not be seen as a stressor since it is not associated with increases in basal plasma C levels (Starzec, Berger, & Hesse, 1983). Chronic forced running does increase basal plasma C levels (Kant, Bunnell, Mougey, Pennington, & Meyeroff, 1983), and thus could be seen as more stressful than a voluntary exercise program. However, since no hormonal measures were obtained in the present study, a stressor will be operationally defined, based on the following discussion, as "an environmental event which results in increases in BP and HR."

The Control of BP and HR.

The major dependent variables in this work are BP and HR. These dependent variables were chosen since they both reflect the endpoints of several physiological processes, and required no invasive procedures. Mean arterial pressure is a function of the output of the heart (cardiac output: CO) and TPR. Direct neural control of TPR is exerted by the sympathetic division of the autonomic nervous system (SNS). Sympathetic nerves innervate the smooth muscle of most peripheral vascular beds, and upon activation result in vasoconstriction of arteries and the arterioles. (Milnor, 1980a). Epinepherine, secreted by the adrenal medulla upon SNS stimulation (e.g., McCarty & Kopin, 1978a), also plays a relatively more sustained but less powerful role in this vasoconstriction (Abboud, 1982).

Cardiac output is a function of heart rate and the volume of blood ejected during systole (stroke volume). HR is modulated by extrinsic sympathetic and parasympathetic stimulation of the sinoatrial node.

Sympathetic stimulation increases HR, while parasympathetic outflow via the vagus nerve slows HR (Berne & Levy, 1977). The baroreceptor reflexes interrelate the control of BP and HR. Receptors in the carotid sinus and aortic arch, sensitive to stretch and gas composition (pH^+, pCO_2), can effect changes in the ratio of sympathetic to parasympathetic stimulation of the heart, and thus compensate for changes in BP by altering HR. Stroke volume is affected by intrinsic factors including myocardial fiber length, myocardial contractility, and ultimately the venous return (Robinson, 1980).

Although this simplified view of the control of HR and BP does not include the numerous feedback and regulatory mechanisms that serve to maintain BP and CO via homeostatic adjustments (see Folkow (1982) or Weiner (1979)), all relevant controlling factors have been mentioned.

The Effects of Stress on the Cardiovascular System: Acute effects.

Stressful stimuli activate the SNS (e.g., Milnor, 1980b), in a response termed the "alerting stage of the defense reaction" (Selye, 1976). Sympathetic nerve activity causes vasoconstriction of renal, mesenteric, splancic, and cutaneous beds (e.g., Cohen & Obrist, 1975), vasodilation of skeletal muscle beds (Abrahams, Hilton, & Zbrozyna, 1964), and release of NE from presynaptic terminals (Weiner, 1979). Additional physiologic consequences of stress are sequelae of sympathetic nerve stimulation on various organs. Increases in HR and CO result from increased nerve activity mediated by beta-adrenergic receptors (Langer, Obrist, & McCubbin, 1979; Gilmore, 1974). Increased CO raises BP directly, and indirectly via autoregulatory constriction of vascular beds (e.g., Guyton, Coleman, & Granger, 1972). Sympathetic

drive to the kidney causes sodium and water retention and the activation of the renin-angiotensin system (Abboud, 1982; Tobian, Janecek, Tomboulian, & Ferreira, 1961), that serves to increase vascular resistance (Abboud, 1974). Stressful events also cause a relatively prolonged release of ACTH, resulting in corticosteroid secretion by the adrenal cortex, which helps to maintain the vascular and pressor responses to NE (e.g., Weiner, 1979). The release of corticosteroids does not appear to habituate with repeated stressors, which can result in increased rates of CA synthesis and release (Fenske, Fuchs, & Probst, 1982).

It is thought that the following sequence of events may occur when no vigorous physical activity is engaged in during stress application. Activation of the SNS results in vasoconstriction of the major visceral beds, vasodilation of the skeletal muscle bed, and an increased CO. These events are presumably designed to prepare the organism for imminent "fight or flight". If the increased blood flow to skeletal muscle is in excess of the metabolic requirements of the muscle, then a local compensatory increase in arteriole resistance will occur via smooth muscle contraction (Langer et al., 1979). This local autoregulation, occurring in less than a minute (Djojosugito, Folkow, Lisander, & Sparks, 1968), serves to dramatically increase resistance to flow in the precapillary bed of skeletal muscle (Folkow, 1982). Since the skeletal muscle bed receives a large percentage of the cardiac output, this response may be a predominant regional influence contributing to increases in total peripheral resistance after stress (Forsyth, 1971). This autoregulatory increase in resistance, following an initial vasodilation, may be a predominant factor in the

aforementioned effects of SNS activation on increasing BP (Folkow & Neil, 1971).

Chronic effects.

All of the above-mentioned effects share a final common pathway in initiating an increase in TPR, and thus BP. Constant periods (1 to 2 weeks) of increased BP have been shown to result in widespread and long-term alterations in the structure of precapillary vessel walls (Bevan, 1976; Lundgren, Hallback, Weiss, & Folkow, 1974). These structural adaptations, involving smooth muscle hypertrophy and hyperplasia (Folkow, 1982; Meehan, 1983), act to reduce the wall/lumen ratio, thus increasing TPR and further augmenting the contractile response (Folkow, 1978) and maintaining the increased pressure. Although these structural changes can also be reversed by chronic hypotension (Weiss, 1974), these vascular changes caused by repeated stress are generally recognized (e.g., Folkow, 1982) as the pathophysiological outcome of stress-induced HT.

With repeated stress, other important adaptations of systems affecting the cardiovascular system occur. Hormonal responsiveness is altered: although resting CA levels and rates of adrenal CA synthesis may be elevated after repeated stress, plasma levels of CA are lower in the presence of a familiar stressor compared to levels upon first presentation of the stressor (Kvetnansky, McCarty, Thoa, Lake, & Kopin, 1979; Kvetnansky & Mikulaj, 1970). Increases in C levels, however, do not appear to show this adaptation (Kvetnansky et al., 1979), and may actually increase further upon repeated presentation of certain stressors (Kant et al., 1983).

Under repeated stress and the associated chronic increase in TPR, the heart must work harder under this increased pressure load. Although the cardiac output may return to prestress levels (Jern, Sivertsson, & Hansson, 1981; Messerli, Frohlich, Suarez, Reisen, Dreslinski, Dunn, & Cole, 1981), other cardiac adaptations may involve hypertrophy of the left ventricle, which in the absence of circulatory compensation makes for a less efficient heart reflected by lowered stroke volume and increased HR for a given cardiac output (Gilmore, 1974).

Effects of Exercise on the Cardiovascular System: Acute effects.

During physical exercise, several hemodynamic and metabolic alterations occur to keep pace with the increased energy demands of skeletal muscle. The SNS is activated, resulting in tachycardia and an increased CO delivered to the exercising muscle. TPR decreases (Groen, Hansen, Herrmann, Schafer, Schmidt, Selbmann, Uexkull, & Weckmann, 1982), due largely to vasodilation in skeletal muscle beds (Milnor, 1980b). BP may either remain unchanged or rise with moderate to high levels of exercise (Cohen & Obrist, 1975). Metabolic effects include an overall increase in metabolic rate, resulting in more rapid catabolism of CA (Harri & Kuusala, 1981). Thus, the acute responses to exercise resemble those elicited in the defense reaction. However, the major difference in the physiologic response is a shunting of the increased blood flow to muscle and a resultant overall decrease in TPR.

Chronic effects.

Exercise training has been shown, though not consistently, to have a modest effect in reducing BP in humans and in both normotensive and hypertensive animal models (see Scheuer & Tipton, 1977 for review).

Most established effects of exercise training are manifest on the heart. No consistent changes in CO or TPR due to chronic exercise have been recognized (Scheuer & Tipton, 1977).

The best known cardiovascular adaptation to exercise is a slower resting heart rate, referred to as training-induced bradycardia (TIB: Steinhaus, 1933). The cause of TIB remains controversial. Most investigators believe TIB to be due to either alterations within the autonomic nervous system, to a reduction in the intrinsic HR, operationally defined as the HR after complete autonomic blockade (Jose, 1966), or both. The alterations in the autonomic nervous system involve an increase in parasympathetic and/or decrease in sympathetic drive to the heart with training (Scheuer & Tipton, 1977; Brundin & Cernigliaro, 1975 ; Lin & Horvath, 1972). These effects, particularily the increased tonic parasympathetic activity via the vagus nerve after exercise training, are fairly well established (Scheuer & Tipton, 1977).

The intrinsic changes generally can be seen to lead to the development of a more efficient heart. Stroke volume is increased, myocardial hyperplasia and a general cardiac enlargement occurs (Scheuer & Tipton, 1977), and increased myocardial contractility may occur (Winters, Leaman, & Anderson, 1973), although this latter adaptation has been disputed (e.g., Carew, Dennis, & Covell, 1974). The cardiac hypertrophy, in its pathologic form generally associated with depressed contractile function, is apparently independent of any changes in contractility (Malhotra, Penpargkul, Schiable, & Scheuer, 1981; Schiable & Scheuer, 1981). Regardless of the ultimate cause of TIB, most investigators agree that "a functioning adrenergic nervous system is necessary for the development of..(TIB)" (Lewis, Nylander, Gad, &

Areskog, 1980, p.303).

Effects of Exercise on Stress Responses

Several studies have been conducted in which the effects of exercise and stress are concurrently evaluated. In one study using a forced exercise procedure (Birrell & Roscoe, 1978), rats were trained on a treadmill for 26 weeks. Both these animals and a sedentary group were then exposed to five daily sessions of escapable shock. The exercised subjects showed no detrimental effects, while the sedentary animals showed adverse morphological changes in the ventricular tissue and microcirculation of the heart. In humans, prior physical training has been shown to result in faster recovery of electrodermal response and heart rate after a battery of mental stressors (Keller & Seraganian, 1984). Other subjects, who were trained in meditation and music appreciation, did not show a rapid autonomic recovery after stress, suggesting specificity of the exercise effect. However, since several subjects withdrew from the exercise program, the remaining subjects may have been more able to cope with both the exercise program and the mental stressors. In another study using men (Raab & Krzywanek, 1965), questionnaires assessed the subjects' exercise habits and reactivity to stress. Cardiovascular responses to a mental arithmetic task with sensory interference were then measured. Only data from subjects with extreme scores on the questionnaires were analyzed. No differences in blood pressure were found. The greatest increases in heart rate after stress were found in the subjects who exercised little and reported high emotional reactivity. The smallest changes in heart rates were found in the physically active and emotionally placid group. Although there were many problems with this study, including the use of unvalidated self-

report scales and systematic exclusion of subjects, the results were intriguing.

Thus, prior exercise conditioning may reduce responses to subsequent stress, as these studies suggest. However, this hypothesis is not equivalent to the supposition that exercise can attenuate reactions to concurrent stress. Two more relevant studies have been conducted using concurrent exercise and stress treatments, one involving a voluntary exercise paradigm and the second using the borderline hypertensive rat (BHR: see below). In the latter study (Cox, Hubbard, Lawler, Sanders, & Mitchell, 1985b, in preparation), two hours of daily forced swimming attenuated HT due to chronic shock stress in the BHR. Tail cuff BP's in unanesthetized subjects (see method, Study I) were taken weekly for the 12 week duration of the treatments. The stressor was two hours of 350 predictable one sec tail shocks ranging in intensity from 0.25 to 0.4 mA. Swimming occurred two hours after the stress session. A stress-only group did not receive the exercise treatments, and a maturational control group received neither intervention. From analysis of changes in plasma CA levels after the final stress session, the authors conclude that exercise dampened the SNS response to stress as evidenced by significantly lower levels of NE in the stressed animals given forced exercise as compared to the nonexercised stressed group.

However, it has been frequently postulated that swimming may act as a powerful stressor in itself (Scheuer & Tipton, 1977; Dawson & Horvath, 1970). The results of Cox et al. could be explained as being due to development of cross-tolerance to the shock stress. Experience with the alleged stressor, swimming, may have lessened the perceived intensity of

the shock stressor, resulting in an attenuated stress response. It is also possible that the initiation of the training adaptations attributed to swimming may not have been due to physical exercise per se, but to physiologic alterations associated with the diving reflex and/or chemoreceptor activation (Tipton, 1984). It was also not made clear whether BP of the exercise plus stress group increased over time.

In another study using voluntary exercise (Starzec et al., 1983), normotensive rats were fed a cholesterol supplemented diet and were subjected to daily 50 min sessions of either predictable, controllable footshock (experimental group: E), unpredictable, uncontrollable footshock (yoked group: Y), or no shock (C) for 30 days. A normal diet control group was also included (N = 12 per group). The E group trials were as follows: A lever press was required to avoid or escape a 0.5 sec shock. A correct response resulted in a five min signalled "safe" period. In the absence of a response, shocks were delivered on a variable 60 sec interval schedule. Each rat in the Y group was yoked to an E group subject. The C group was handled each day, but it was not clear if they were also placed in the shock chambers. Half of the animals were allowed access to a running wheel for three hours immediately after the stress treatments, resulting in a 2 (exercise/no exercise) x 3 (E, Y, or C) factorial design. The dependent variable was levels of plasma corticosterone (C) measured immediately after the final stress session.

Stressed animals allowed to run showed lower levels of C than rats not allowed to run. Although the C group showed higher plasma C levels than the normal diet group, shock stress was associated with plasma C levels significantly higher than those in the C group. The authors

conclude that such a program of voluntary exercise can attenuate the corticosterone response to stress. There was no effect of exercise in the handled control group, showing that a program of voluntary exercise apparently does not increase plasma C levels.

Since footshock is commonly used in studies of stress, as exemplified by the above two studies, this type of stressor was also used in the present report.

Effects of Exercise on Stress Responses: Possible Mechanisms

The mechanism(s) of the effects of exercise in attenuating high blood pressure and heart rate due to chronic stress remain uncertain. Exercise could exert its influence at several points along the path leading from the stress reaction to elevated TPR.

One way exercise could block stress-induced HT (referred to as the local autoregulation (LA) hypothesis) is as follows. Exercise results in increased blood flow to muscle, both due to an increased cardiac output and sympathetic vasodilation of the skeletal muscle vascular bed (e.g., Scheuer & Tipton, 1977). Since during muscular exertion the increased flow to muscle is immediately utilized, compensatory autoregulatory constriction and resultant structural adaptations would not occur. This hypothesis would predict that exercise concurrent with stress would be most effective in blocking this nonneurally mediated constriction. If constriction and the resultant increase in BP normally persists for some time after the stressor has been removed, then exercise immediately after stress may be effective by rapidly halting constriction in skeletal muscle beds.

Exercise could also directly attenuate the activity of the SNS in response to stress (Cox et al. 1985). Another study has suggested that the tissue responses to SNS activity may also be reduced by exercise, since injections of NE produced significantly lower increases in BP in athletic than non-athletic subjects (Pavlik & Frenkl, 1975). Further, exercise could lower rates of synthesis of adrenal CA (Tipton, 1984), effectively reducing the adrenergic stimulation on the heart and vasculature. Exercise could also modify other aspects of the consequences listed previously of SNS activation in response to stress, such as an increase in the parasympathetic and decrease the sympathetic drive on the heart (Ekblom, Kilbom, & Soltysiak, 1973; Scheuer & Tipton, 1977), thus blocking the increase in CO and HR due to stress; and/or a halt in the sympathetic effects on the kidney, since exercise training has been associated with lowered plasma renin levels (Convertino, Brock, Keil, Bernauer, & Greenleaf, 1983), and SNS activity can stimulate renin release (Davis, 1974).

Exercise could cause intrinsic cardiac changes such as increased stroke volume and contractility (Winters, et al., 1973; Lewis et al., 1980), thus reducing HR and BP at a given level of SNS activity. Exercise may also attenuate stress-induced glucocorticoid release (Starzec et al., 1983), since glucocorticoids may have a modulatory effect on adrenal CA synthesis (e.g., Matlina, 1984) and/or reduce BP by lowering body weight (e.g., Blackburn, 1978), probably via a reduction in plasma volume (Tipton, 1984). Finally, by lowering HR and pressor responses to stress, exercise could decrease the perceived physiological activation and thus not cause further emotional activation based on perception of autonomic arousal.

Thus, there are two possible general mechanisms to explain the effects of exercise: 1) exercise could attenuate stress-induced HT by directly reducing the SNS response to stress, or 2) by attenuating aspects of the sequelae to SNS activation.

Models of Stress-induced Hypertension

Hypertension has been induced by various means such as alterations of the social environment in mice (Henry, Stephans, & Santistaban, 1975; Henry, Meehan, & Stephans, 1967), operant conditioning procedures involving shock in dogs (Langer et al., 1979), primates (Forsyth, 1971; Goldstein, Harris, & Brody, 1977), and rats (Buchholtz, Lawler, & Barker, 1981), shaking of cages (Bunag, Takeda, & Riley, 1980), and by motion, noise and flashing lights in rats (Smookler & Buckley, 1969). However, the HT induced in the latter two studies have been only in the borderline hypertensive range, and in the remaining studies was not maintained in the absence of stress.

Use of the Borderline Hypertensive Rat

The use of animal models genetically predisposed to develop normotensive pressures may not be the ideal model in investigating the effects of stress. However, since a certain degree of genetic predisposition may be required (Lawler, Barker, Hubbard, Cox, & Randall, 1981; Freidman & Iwai, 1977), one obvious model is the spontaneously hypertensive rat or SHR (Okamato, 1972). The etiology of spontaneous hypertension in these animals is thought to be similar to that of human essential hypertension, in that both involve an increased CO and normal TPR followed by a chronically elevated TPR with normal CO (e.g., Smith & Hutchins, 1979). However, the SHR develops HT at such an early age, and

with such consistency, that it is also inappropriate for studies involving chronic stress. What is required in an appropriate animal model of stress-induced HT is development of sustained HT only after chronic stress, as in borderline HT in humans, and pathophysiological adjustments similar to that seen in human populations.

A more appropriate subject for studying factors affecting the interactions between stress and genetic predisposition to develop HT has been used by Lawler and colleagues (1981). These rats were produced by mating female SHR with males of its normotensive progenitor strain, the Wistar-Kyoto or WKY. These first generation homozygous offspring are genetically predisposed to develop borderline hypertension as adults (140-160 mmHg systolic BP). Prolonged and pronounced HT has been induced in the BHR by the powerful "psychological" stressor of shock-shock conflict (Lawler et al., 1980; Lawler et al., 1981). In this paradigm (similar to discriminated avoidance) animals are shocked for not responding during a signal preceding shock, and also receive a briefer shock when an effective response is made. Unsignalled, predictable shock has also consistently resulted in HT in this model and thus appears to be a satisfactory stressor (Cox et al., 1985). Thus, the use of this paradigm appears to have much potential for investigation of the interactive effects of stress and exercise.

Use of Voluntary Exercise

Nearly all studies employing physical training in animals have used forced treadmill or swimming. Although the main advantage of these treatments is that the amount and magnitude of exercise is controlled, they constitute stress, in themselves, for the animal (Cox, Hubbard,

Lawler, Sanders & Mitchell, 1985a). This may contribute to the lack of effect of exercise training on BP in animals. In fact, in one study (Suzuki, Oshima, & Higuchi, 1979), forced exercised SHR's had higher pressures than their nontrained controls. When assigned to a voluntary exercise group, the trained animals had pressures approximately 30 mmHg lower than their controls. Especially when concurrently evaluating the effects of stress on cardiovascular parameters, the alternative paradigm of voluntary running wheel exercise is more appropriate. The only study which has used this paradigm to attenuate the effects of stress was that of Starzec et al. (1983).

The present studies are designed to establish the effects of various stressors on BP and HR, and the effects of voluntary exercise in attenuating HT and TC induced by these stressors in an animal model of stress-induced HT. These studies, when combined with future work evaluating the mechanism of the effect of exercise on HT, have the potential of providing an effective model for investigating how life stress can result in HT and TC in populations genetically at risk, and how one possible treatment intervention can block these pathophysiological outcomes.

STUDY I

This experiment addressed whether stress-induced hypertension and tachycardia can by attenuated by voluntary running wheel exercise in the BHR. A second aim was to see if having control over the stressful stimuli of shock presentation would result in reduced levels of BP and/or HR than having no such control over shock.

Method

Subjects

Eighteen male and 18 female BHR rats from eight litters of SHR male-WKY female matings were group housed (2-3 animals/cage), from weaning on Day 21 until 16 weeks of age, and maintained in a colony room with other rats on a 12:12 light:dark cycle with food (Purina Rat Chow) and water available ad lib. Eight additional animals (4 male, 4 female) from three litters, maintained under the same conditions, were left undisturbed until BP measurement at 25 weeks of age. These BHR subjects served as controls for the effects of maturation.

The original design called for the use of all male animals, but due to a lack of sufficient male offspring, both males and females were used as subjects.

Experimental Design

Avoidance Training.

Before subjects could be assigned to the stress conditions in which control over shock presentation was manipulated, a training period was given to facilitate the acquisition of the avoidance response required

to control shock delivery. To control for the possible effects of this avoidance training, all experimental animals, except for the maturational control subjects, were exposed to a discrimination paradigm similar to that used by Lawler et al. (1980) for eight daily 50 min sessions. In this procedure, the animals were placed in an operant chamber (Gerbrands Model D) inside a sound-attenuating box. After five sec, a stimulus light (CS) was presented. A lever press by the animal, during or before this period reset the clock to the start of the pre-CS period and resulted in avoidance of a 0.6 mA, one sec scrambled shock delivered via the grid floor. A failure to respond during the five-sec CS period resulted in delivery of inescapable shock. CS and shock presentation were computer controlled. A learning score was calculated for each session based on the number of effective responses made by the animal. This score was equal to the total number of shocks received (NSHK) divided by the total number of effective responses + NSHK, multiplied by 100 to remove the decimal point. A score of 0 would indicate avoidance of all shocks by the animal, and a score of 100 failure to avoid any shock. For the two days following avoidance training, BP and HR measurements were again taken on all animals, now 17 to 18 weeks of age.

Stress and Exercise Treatments.

Following avoidance training, subjects were randomly assigned to one of six groups, but each group had to have three males and three females. A maximum of three offspring from a given litter were assigned to a given experimental group to control for a potential effect of the preweaning environment and genetic differences on the outcome variables. Analysis of variance showed that these groups did not differ ($p > .10$)

in the total number of shocks received, mean learning score during the last avoidance session, or BP and HR before or after avoidance training. Three levels of stress and two levels of exercise resulted in the following six groups: shock-shock conflict- exercise, yoked-exercise, handled-exercise, shock-shock conflict-no exercise, yoked-no exercise, and handled-no exercise, with six animals per group.

The conflict condition was identical to the avoidance training procedure, with the exception that an effective lever press resulted in a brief (0.2 sec) shock. The yoked groups received the same frequency and patterning of shocks as the conflict animals, but had no control or signal of impending shock delivery. The "handled" groups were placed in the chambers for the same length of time as the shocked animals, but no stimuli were delivered. The "handled" groups were included as a control for the effects of daily handling during transfer to the shock chambers. These stress protocols were followed for seven weeks, five days a week. Each session lasted 40 minutes. During each session, all of the animals in a group were run simultaneously, after which the grid floor and trays of the chambers were cleaned. All stress sessions occurred between 8 AM and 3 PM.

The order in which a given "conflict" animal was yoked to a given control was rotated daily. The daily order in which the experimental groups received the stress treatments was randomized to control for circadian effects. Within each group, the actual shock chamber the animal was placed in was rotated daily among the subjects, since the shock generators, although of the same model, had slight variations in current output.

The exercise treatment consisted of free access to a 14 inch diameter running wheel (Wahmann LC-34), equipped with a counter, attached to the animal's home cage via a four inch segment of 2.5 inch diameter plexiglas tubing. The counters were checked daily and distance run in kilometers (km) was calculated on a weekly basis for each animal. On four days during the final two weeks of treatments, the amount of running in the first hour immediately after daily stress sessions was observed. Half of the subjects from each exercise condition were allowed to exercise in this manner. The remaining animals were housed in identical cages with no wheels attached.

Three days after the conclusion of treatments, blood pressure was taken from all animals (now 25 weeks of age), who were then housed in pairs in cages without running wheels and left undisturbed for four additional weeks. Final BP and HR's were then taken on all animals.

Blood Pressure Measurement

All blood pressure observations were taken by the "tail cuff method", which tends to generate higher readings than direct BP measurement, with a positive correlation between these methods of 0.80 to 0.90 (Bunag, 1973). Animals were prewarmed for 10 min at 36°C, then placed in a customized restraining device inside a sound-attenuating chamber maintained at the same temperature. An inflatable cuff was placed around the tail and a photosensitive cell and light source placed distal to the cuff. The photocell is sensitive to systolic pulsations in the tail artery. These pulsations triggered a cardiotachometer, providing a simultaneous record of heart rate. Cuff pressures are automatically increased and decreased for each BP recording.

Heart rates were noted just prior to an inflation-deflation cycle. At least 20 sec elapsed between successive cuff inflations. Systolic BP was recorded as the cuff pressure coincident with the return of the pulse pressure.

The mean of at least three artifact-free tracings were taken as the BP and HR of that animal. On occasion, body movement during the recording period necessitated more than three readings. The measurement procedure lasted about 10 min. All BP measurements were taken between 12 and 4 PM. The experimenter was blind as to the experimental condition of the animal being measured. After baseline BP and HR were taken at 16 weeks of age, all experimental animals were individually housed in opaque polypropylene cages (46 x 24 x 20 cm).

Results

Avoidance and Conflict Learning

Analysis of learning scores on the final day of avoidance training showed that the procedure employed resulted in very poor acquisition. Only five of the 36 animals received learning scores below 95 during this session, four of these five receiving scores between 75 and 95. When group data were analyzed, no group had learning scores significantly different from 100 in that the mean plus the standard error of the mean (S.E.M.) was greater than 100. Of the five animals that did have a learning score under 95 on the final day of avoidance training, three had been randomly assigned to the conflict group. Two of these animals failed to maintain even minimal (mean learning score under 99) responding when placed in the conflict paradigm. The animal with a score on the final day of avoidance training under 95, which had

been assigned to the conflict group, was the only animal to show any maintenance of responding (mean learning score of 85) during the seven weeks of conflict.

Because of these results, the type of stress experienced by the animals was not that originally intended, but after exclusion of the above animal (S-NE group), it could be redefined as follows. The conflict group effectively received signalled predictable shock, either with exercise (S-E), or without exercise (S-NE). The yoked group received unsignalled shock as originally designed, with (U-E) or without (U-NE) exercise. The "handled" group received no shock, also with (H-E) or without (H-NE) exercise. Figure 1 presents a diagram of the redefined experimental design. Numbers within parentheses in each cell refer to the number of subjects per condition.

Running Wheel Activity

All animals in the voluntary exercise conditions used the wheels extensively each day. There were, however, differences in the amount of running among the stress groups (group x time repeated measures: $P(F = 7.06) < .01$, $df = 2,71$), with the "handled" subjects running more than the animals receiving signalled shock (Newman-Keuls: $p < .05$). In figure 2 the amount of running, expressed as kilometers per week per animal, is shown for each of the exercising groups over the course of the seven week treatment period. The distance run increased significantly over time in all groups as determined by linear trend analysis ($P(F = 18.84) < .01$, $df = 2,71$). The "handled" groups ran an average (mean $\pm$ S.E.M.) of 41.81 $\pm$ 7.97 km/wk, the U-E group 36.66 $\pm$ 5.23 km/wk, and the C-E subjects 32.06 $\pm$ 6.46 km/wk.

Running rates were estimated by timed (10 min) observations in ten animals over the course of the experiment. The mean rate of running was 17.2 ± 4.8 meters/min.

From observations made on four days during the final two weeks, about 12% of the total running on stress treatment days occurred in the first hour after being returned to their home cage. Thus all animals ran more in this first hour after the treatments than the average number of runs per hour on that day ($P(F = 4.90) < .05$, $df = 2,90$). All three exercising groups showed this effect, with no differences among the groups.

Blood Pressure and Heart Rate

Table 1 presents mean (± S.E.M.) BP and HR for all experimental groups during the course of the experiment. These results, summarized below, are presently graphically as mean change scores from baseline in Figure 3.

Except for results from the avoidance training and follow up measurements, all analyses were based on change scores from initial baseline measurements to readings taken after the termination of all treatments.

Avoidance training.

The avoidance training procedure resulted in a small but significant rise in BP ($P(t = 2.85) < .01$, $df = 70$) from an initial baseline (± S.E.M.) of 157 ± 2.6 to an average pressure of 168 ± 2.4. Heart rate decreased ($P(t = 2.52) < .05$, $df = 70$) from a mean of 447 ± 9.2 to 415 ± 9.0 after avoidance training in the experimental animals.

Non-exercised animals.

Blood pressure

Planned comparisons revealed that there was a significant overall increase in BP in the non-exercised animals as a consequence of stress ($P(t = 9.13) < .01$, $df = 32$). BP increased significantly from baseline values in all three stress groups. Further, these increases were not significantly different among the stress types, although the handled and unsignalled shock groups showed a greater, although not significant, rise in BP than the S-NE animals (Means $\pm$ S.E.M.: H-NE 45.5 $\pm$ 11.6; Y-NE 42.7 $\pm$ 5.3; S-NE 26.6 $\pm$ 11.2). That these changes were due to stress and not maturation is indicated by the fact that initial baseline measurements of BP of these experimental animals (153.3 $\pm$ 3.2) were not significantly different from those of the MC group, the mean of which was 155.8 $\pm$ 15.3 mmHg when measured at 16 weeks of age. Also, the increase in blood pressure from baseline readings to termination of treatments was significantly greater in the non-exercised subjects (35.7 $\pm$ 5.7) than the changes in BP of the maturational control animals (3.4 $\pm$ 5.6) measured at the same age of 25 weeks ($P(t = 2.72) < .01$, $df = 41$). The increase from baseline in BP in the experimental animals was still significant after the four week recovery period ($P(t = 5.10) < .01$, $df = 32$).

Heart Rate.

Although the increase in heart rates from baseline was not significant for any of the stress types when analyzed independently (t-test), a planned comparison revealed that this increase was significant ($P(t = 2.26) < .05$, $df = 34$) when data were pooled for all groups. Further, this increase in HR after all treatments in the sedentary

animals (39.0 ± 10.4) was significantly greater than the change in HR (-6.2 ± 12.8) in the MC group ($P(t = 3.04) < .01$, $df = 23$). The HR changes in the stressed animals were not long lasting, as shown in a return to near baseline values in all groups four weeks after termination of treatment.

Exercise trained subjects.

Blood Pressure.

Results for the subjects allowed to exercise revealed different cardiovascular responses. Although BP did increase from baseline to the termination of treatments when the three exercise groups were combined, ($P(t = 2.61) < .05$, $df = 34$), in the H-E group this increase was not significantly different from zero (6 ± 14.8), or from the change in BP over time in the MC group (t-test).

Heart Rate.

Heart rates in the exercise groups, in contrast to the sedentary groups, decreased after stress ($P(t = 4.11) < .01$, $df = 34$), with no differences in the degree of bradycardia among the three groups. Only the U-E group did not show a significant decrease in HR.

Effects of voluntary exercise.

In order to clarify the effects of exercise on BP and HR, two-way (stress x exercise) ANOVA's were conducted on the change scores in blood pressure and heart rate from initial baseline measurements to recordings made after the termination of all treatments. No effects of gender were found on changes in BP or HR from baseline to the termination of treatments in the experimental animals. The variances associated with these analyses were also found to be homogeneous ($F' > .10$ in all cases).

Therefore, the two-way ANOVA did not include gender as an independent variable. Voluntary exercise significantly attenuated the increases in both blood pressure ($P(F = 4.86) < .05$, $df = 1,29$) and heart rate ($P(F = 18.19) < .01$, $df = 1,29$) from initial values of the experimental subjects.

No significant main effect of stress was obtained for BP or HR. Although the stress by exercise interaction in this analysis was not significant for BP or HR, post-hoc comparisons among the three stress groups revealed that the "handled" subjects showed the greatest exercise effect on BP (H-E vs. H-NE, Newman-Keuls: $p < .05$), while the unsignalled shock group showed a trend in this direction (U-E vs. $p < .10$). The effect of exercise on HR was significant in each of the stress groups (Newman-Keuls: $p < .05$). Both these comparisons and the overall effects remained valid when the amount of running in each stress group was adjusted by using the total distance run as a covariate.

Discussion

The major finding of this study was that voluntary exercise attenuated increases in BP and HR due to chronic stress in the BHR. This effect of exercise on BP was most robust in the handled control groups. This study is the first demonstration of this effect of voluntary exercise on either HR or BP.

In the non-exercised animals, blood pressure and heart rate of the BHR subjects increased due to three types of daily stressors. The fact that the stressed animals had higher pressures and heart rates than the maturational controls suggests that these increases were due to the

stress treatments per se, and not to general environmental conditions or aging.

These results are consistent with results of other studies showing increases in BP and HR in BHR rats following either shock-shock conflict (Lawler et al., 1980; Lawler et al., 1981) or unsignalled shock (Cox et al., 1985). The decrease in HR following avoidance training may reflect a decrease in cardiac output shown to occur after relatively short-term shock stress (Forsyth, 1971). Voluntary running wheel exercise not only blocked the stress-induced increases in HR due to daily stress, but in fact resulted in decreases from the initial values.

In this study it was intended that shock-shock conflict be incorporated as one of the stressors. However, only one animal acquired the avoidance response and maintained responding. These procedures differed from previous studies by Lawler et al. (1980, 1981), in which the conflict paradigm was successfully employed. In this earlier work, animals were required to turn a wheel to avoid tail shock, as opposed to the present study in which a lever press was necessary to avoid foot shock. The response of wheel turning is more similar to the observed escape-directed behaviors seen during shock in these animals, namely running or climbing, than to the response of bar pressing. It involves a preparatory escape movement of forepaw extension. Thus, the operant response of bar pressing may have been relatively incompatible with this species-specific defense reaction engendered by shock (Bolles, 1970). In the procedures of Lawler et al., animals were also punished if they failed to maintain an adequate level of responding, and a longer (15 days) avoidance training period was used with a 10 sec instead of a 5 sec CS and pre-CS period. Any of these modifications may have

contributed to the failure of the subjects to acquire and maintain adequate responding in this experiment. Nevertheless, by excluding the animal that retained the avoidance response, the stress received by this group effectively became signalled shock.

The group which received only eight days of avoidance training and no further shock (H-NE group) also showed significant increases in BP and HR. These results were not expected, as this condition was included as a control group. Thus, the stressor was apparently not under experimental control. These changes were thus presumably a consequence of daily handling and/or placement into chambers previously associated with footshock. In fact, all stressors used in this experiment involved multiple and confounded treatments, which could be additive.

The signalled and unsignalled shock groups also included social isolation of the animals and daily handling and placement into the shock chambers. The "handled" groups were also socially isolated, and were placed into operant chambers previously associated with footshock, thus perhaps experiencing threat of shock delivery. Presumably, the conditional association of the operant chambers with footshock in the "handled" animals might extinguish over the relatively long time period of this experiment. This contention is supported in a study in which the behavioral response to a CS previously paired with shock extinguished over time when presented alone (Millenson & Dent, 1971). All animals were placed in the same set of operant chambers: thus the "handled" animals were also exposed to olfactory stimuli given by their shocked conspecifics. Rats, with well developed olfactory systems, are very sensitive to these putative pheromones, known to be released during stress (Mackay-Sim & Laing, 1981). Odors from stressed rats have been

shown to affect the activity of non-stressed conspecifics (Mackay-Sim, 1980), and may affect other behavioral and physiological responses as well. In fact, one recent study (Fanselow, 1985) reported the induction of analgesia in rats due to placement into an environment where conspecifics had recently been shocked.

Although social isolation alone has not been shown to increase BP in the BHR (Lavler et al., 1980), its effects on HR in this strain of animals is not known. Continuous isolation of normotensive Wistar rats for just five days has been shown in one study (Gardiner & Bennett, 1977), to result in significant increases in both HR and tail cuff BP. Thus, isolation may have contributed at least in part to the HR and BP increases observed in the non-exercised subjects. In addition, the BP measurement procedures themselves, involving heating and restraint of the animal, may have had an exacerbating effect on "basal" BP and HR, or a synergistic and confounded effect with isolation. However, presumably this effect would be equal in all groups.

Although the effects of forced exercise on decreasing HR is well documented, exercise has only modest effects on reducing BP in otherwise unstressed subjects (e.g., Blomqvist & Saltin, 1983; Scheuer & Tipton, 1977). Thus an effect of exercise may be more easily demonstrable when given concurrently with stress. This effect of exercise on HR is especially noteworthy since it occurred in animals subjected to stress treatments that resulted in HR increases in the absence of exercise. However, it was found in the present study that the effects of both exercise and stress on HR were not long lasting. There was a return to near baseline values four weeks after termination of all treatments. In contrast, blood pressures remained elevated from baseline after recovery

due to daily stress. This elevation has been shown to persist as long as 12 weeks after termination of conflict shock stress in the BHR (Lavler et al., 1981; Lavler et al., 1984). However, the effect of exercise on the cardiovascular system is often lost within a few weeks after cessation of training (Scheuer & Tipton, 1977). The different time courses for recovery of BP and HR due to chronic stress may reflect different underlying mechanisms for the physiological adaptations involved. One explanation would involve permanent structural adaptations in the smooth muscle of arterioles as a result of the increased BP due to stress (Folkow, 1982; Bevan, 1976), in contrast to the HR increases due to stress reflecting a reversible increase in resting sympathetic and/or decrease in parasympathetic tone. This result also implies that dynamic changes in physiological setpoints are more likely mechanisms to explain changes in HR due to stress and exercise than are structural changes, such as cardiac hypertrophy and changes in myocardial fiber length. This view is in general agreement with the consensus on the cause of training-induced bradycardia (Tipton, 1984; Blomqvist & Saltin, 1983). However, since the subjects were group housed during these four weeks, the changes in BP and HR are potentially confounded with this change in housing condition.

It is possible that the attenuation of stress-induced HT and TC in the exercised subjects reflected not an effect of exercise <u>per se</u>, but the ability to enter into another area from the home cage. This possibility is addressed in the second experiment.

In this first study it was also found that voluntary exercise was effective in attenuating HT induced by daily stress. It was not expected that exercise would lower BP in some groups and raise BP in

others, so a stress by exercise interaction was not predicted. Based on post-hoc tests, the exercise effect was most pronounced in the "handled" group, even when adjustments were made for differences in the amount of running among the groups. While the "handled" animals with no access to running wheels showed a highly significant 45 mmHg increase in BP from baseline, the blood pressure of those able to run showed a non-significant 6 mmHg increase as a consequence of stress. Since voluntary exercise had no effect in the signalled shock group and only a marginal effect in the unsignalled shock condition, this result implies that voluntary exercise is not effective in reducing HT induced by all stressors, but rather may be most effective in countering "psychologically" induced hypertension such as "handling"/threat of shock, as opposed to more "physical" stressors such as high density footshock.

There are three possible explanations for why exercise was effective only in attenuating stress-induced HT in the "handled" groups. First, this finding could represent Type I error. The overall effect of exercise in attenuating stress-induced HT was significant ($p<.05$), and only when the analysis was collapsed over the independent variable of sex. Second, it is possible that the attenuating effect of exercise on HT was not seen in the chronically shocked animals, since during shock the animals engaged in vigorous escape directed activity, while the rats in the "handled" condition generally remained immobile. This activity in the shocked animals may have partially blocked the locally mediated constriction in skeletal muscle beds that exercise is presumed to reduce according to the LA hypothesis. However, this explanation presupposes that this type of muscular activity would be sufficiently intense to

utilize the increased blood flow to muscle, and that autoregulatory constriction can continue after removal of the stressor.

A third explanation is that exercise may only be effective in blocking the hypertensive effects of what are commonly thought of as relatively mild stressors (e.g., "handling"). The shocked animals received a total of about 7400 shocks, while the "handled" groups received no shocks during the period in which exercise was available. Voluntary exercise, therefore, may not be able to counteract such a long term intense stress as that received by the shocked animals.

The animals that were shocked ran somewhat less than the "handled" subjects, paralleling the phenomenon of shock-induced depression of spontaneous motor activity (Weiss, Bailey, Pohorecky, Korzeniowski, & Grillione, 1980). This phenomenon, which was shown to occur after an acute (one hour) treatment with intense (4 mA) inescapable shock, may explain the observed differences in the amount of running in the exercising groups to be due to the effects of shock stress. The acute increase in running just after stress may reflect a discharge of the presumed emotional activation due to the preceding stressor.

Study I was not designed to eliminate alternative hypotheses about mechanisms by which voluntary exercise led to attenuation of stress-induced HT and TC. A pilot experiment was conducted to see if one prediction of the LA hypothesis would be indirectly supported (see appendix). According to this hypothesis, locally mediated vasoconstriction would occur in skeletal muscle beds if physical activity is not engaged in during periods of increased BP. In order for physical activity, i.e., voluntary exercise, to be able to dissipate

this postulated constriction, TPR would have to remain elevated after the animal has been removed from the shock chamber and returned to the home cage, at which time the animal has an opportunity to run. Since no simple and valid measurement of TPR was available, in this study BP was measured directly before, during, and after a stress session.

The second experiment was designed to indirectly test another prediction of the LA hypothesis, that exercise immediately after stress would be more effective in attenuating increases in BP and HR than exercise prior to stress, as well as to replicate and extend the findings from Study I.

STUDY II

The specific aims of the second study were as follows:

1) to replicate the effect of freely available voluntary exercise on attenuating stress-induced HT and TC in the BHR.

2) to delineate in more detail the time course of the changes in BP and HR.

3) to determine whether the increases in BP in the "handled" group in Study I were due to placement into an environment previously associated with shock, daily handling and placement into a different environment, or social isolation.

4) to address whether the attenuation of increases in BP in the exercise groups in the first experiment was due in fact to physical exercise, or to the ability to enter another area from the home cage.

5) to see if the temporal relation between application of stress and exercise had significant consequences on BP.

6) to see if one prediction of the local autoregulation hypothesis, that exercise would be more effective if engaged in immediately after stress than prior to stress, was indirectly supported or not supported.

Experimental Design

To simplify the basis for the experimental conditions in Study II, the groups formed are discussed in terms of the specific aims outlined above. Figure 4 presents the design in a schematic diagram.

In order to determine what aspects of the "handling" procedure resulted in HT and TC in Study I, three types of stressors were used.

One stressor, threat of shock (NS group), involved medium density inescapable footshock for one week, followed by five weeks of very low density shock (see method). A total of 25 shocks were given during these five weeks, presumably not enough to elicit HT and TC in themselves (i.e., if given acutely), but sufficient to maintain a constant threat of shock delivery. Thus, this stressor was expected to be more "psychological" than physical in nature. This stressor should reliably elicit HT and TC in the absence of exercise, since it is similar to the "handling" paradigm used in Study I, with some additional shocks given. The second stressor involved daily transfer and placement into operant chambers as above, but no shocks were delivered at any time. This group (NH) received "handling" and no further stress. To control for these daily manipulations, a third stress group (NN) was also included which was not treated except for weekly BP and HR recording.

Since some of the animals in these stress groups also had access to a running wheel (this requires one animal per cage), all of the above animals were socially isolated and had wire mesh chambers attached to their home cage to allow comparisons to be made. To determine any effects isolation alone may have on BP and HR, another group of BHR's (NNG) remained group housed throughout the experiment. These animals also served as maturational controls.

To see if the availability of another area other than the home cage had any effect on BP or HR, some animals were socially isolated but did not have another area to enter from the home cage and were otherwise undisturbed (NNI). Comparisons made among the NN, NNI, and NNG subjects provided an evaluation of the effects of social isolation and the

ability to enter another area from the home cage on BP and HR.

To provide a more refined examination of the effects of exercise found in the first study, one group of subjects given threat of shock stress were allowed continuous access to a running wheel (CS), while another group had free access to a wire mesh chamber (NS). Half of the animals subjected to daily "handling" had continuous access to a running wheel (CH), and half had access to wire mesh chambers (NH). These four groups provided a test of the effect of exercise in attenuating stress-induced HT and TC.

To evaluate whether exercise would be more effective in blocking stress-induced increases in BP and HR if engaged in immediately after stress, two additional groups were included. Both of these groups were subjected to the stressor most likely to result in HT and TC, namely, threat of shock. One of these groups (IS) could only engage in running wheel exercise for one hour immediately after stress (IS), while the other (PS) could only engage in exercise for a period of 90 min several hours prior to the daily stress session.

The length of time that exercise was available allowed a rough equalization of the actual distance run between these two limited exercise groups. This length of time was used since two hours of forced swimming (Cox et al., 1985) and three hours of voluntary running wheel exercise (Starzec et al., 1983) was sufficient in previous studies to show an effect of exercise. Since this running occurred both before and after the six daily stress treatments, a time period under three hours was chosen so as to avoid disturbing the animals near a change in their daily light:dark cycle.

To summarize, this design addressed four questions: 1) Was the effect of voluntary exercise on attenuating stress-induced HT and TC replicable. (A comparison of the continuous exercise groups (CH + CS) with the similarly stressed non-exercised animals (NH + NS)). 2) What aspect of the "handling" procedure in Study I was necessary and sufficient to cause HT and TC. (Comparisons among the NH, NS, and NN groups, and an evaluation of the effect of social isolation (NNI vs. NNG)). 3) Is the temporal relation between exercise and stress important for changes in BP and HR. (Comparing groups IS and PS). 4) Does having another area accessible from the home cage result in effects similar to those seen when a running wheel is available. (A comparison between groups NN and NNI).

BP and HR's were obtained weekly from all animals by the tail cuff method under light ether anaesthesia. BP measurements using this method have been shown to correlate highly with direct BP readings in awake animals (Borkowski & Quinn, 1983), although ether administration is a known stressor. Advantages of ether use include rapid determinations of systolic BP, and no active struggling or preheating of the animal. When measured in awake animals, the variability in the degree of struggling and length of time in the heated apparatus may have contributed to differences in the BP readings among the animals. It was hoped that this method would result in a significant decrease in the variability of BP and HR readings within an animal.

Method

Subjects

The subjects for this study were 48 male BHR's from 13 litters of SHR male-WKY female matings, group housed (4 animals/cage) from weaning on Day 24 until 10 weeks of age and maintained in a colony room containing other rats on a 12:12 light:dark cycle with food and water available ad lib. Procedures began at this earlier age, relative to Study I, since it was thought that cardiovascular responses would be similar to those at 16 weeks as long as stable adult levels (seen at 8 weeks of age) had been reached.

Subjects were randomly assigned to the experimental conditions, with the precondition that no more than three offspring from a given litter were assigned to the same experimental group.

Blood Pressure and Heart Rate Measurement

Blood pressures and heart rates were taken weekly (except in the NNI and NNG subjects - see below) from baseline values prior to the treatments (Week 0) through Week 5 by the tail cuff method as described in Study I, with the following exceptions. Light ether anesthesia was given as follows. The animals were placed into a glass jar containing ether fumes. Once the animal was anesthetized (5 to 10 min), recordings were made until the animal awakened (3 to 10 min) and moved its tail out of the tail cuff. Occasionally, the animal would awaken before three recordings could be made. In this case, the animal was reanesthetized and the procedure repeated. The median of three consecutive recordings immediately prior to awakening was taken as the BP of the animal.

Median, as opposed to mean, pressures were scored to reduce the effect of occasional extreme values.

Analyses revealed that using the median instead of the mean did not significantly alter any differences among the experimental groups. Since a cardiotachometer was not available for this study, the HR (beats/min) was taken to be the number of systolic pulsations in the tail artery found in a three second period just prior to the recording of the median BP value, multiplied by 20. These pulsations were measured by the tail cuff method (see Study I) Cuff pressures were increased every 10 to 30 sec. The NNI and NNG groups had measurements taken every three weeks, from Week 0 through Week 9. All measurements were taken at least three hours after any stress treatment (between 1 and 6 PM). After baseline recordings were taken at 10 weeks of age, subjects were then introduced to their respective home cage environments (isolation, running wheel, etc.), and treatments began three days later.

To allow some determination of the effects of ether anesthetization on BP and HR, recordings of all subjects were also taken while awake (see Study I for methods) three days after the termination of treatments (Week 6).

Stress Treatments

The animals exposed to threat of shock (IS, NS, PS, CS) were treated as follows: During the first week (Mon-Fri), the animals were placed into operant chambers as in the first study. During the 50 min session, they received one sec pulses of random uncontrollable shock delivered via the grid floor (0.4 mA, scrambled) with a median intershock interval of four min. Thereafter, they were placed into the

chambers as before (50 min sessions) but received only five shocks per week on a random basis for five additional weeks.

All stress treatments, including handling, occurred between 9 AM and 3 PM, and the order in which animals were placed into the chambers were randomized as in Study I. The grid floor was cleaned after every session, and the trays cleaned daily.

The handled treatment (CH, NH) was identical to that of threat of shock except no shocks were delivered at any time.

Exercise Treatments: Continuous exercise.

Animals in the continuous exercise groups (CH, CS) had free access to running wheels from their home cage as described in Study I. Counters were checked daily and compiled weekly for distance run. As in Study I, the distance run in the first hour after stress treatments was noted on four days during the final two weeks of treatments.

Limited exercise.

Animals in the immediate (IS) and prior (PS) exercise groups had a running wheel attached to their home cage, but were able to run only during the times specified. This was achieved by locking the wheels with a metal clip. When running was allowed, the wheel was unlocked and the animal usually then entered the wheel and began running. The water bottle was then moved so as to block a return to the home cage and facilitate running. Occassionally, the animal had to be gently prodded to enter the wheel. At the conclusion of the exercise period, the wheels were locked, water bottles returned, and the distances run noted. To equalize the distance run in the immediate and delayed exercise

groups, pilot observations were made of several Wistar-Kyoto rats to determine how much they ran in the hour immediately after stress, and the amount of time required for an equivalent amount of running, three hours prior to stress. During the experiment, the amount of running prior to stress was closely monitored and duration of wheel access for the IS animals adjusted as necessary. For all but four days of the six week treatment period, the PS animals ran for 90 min and the IS subjects for 60 min. On those four days, both groups ran for 90 min.

Control Groups

Wire mesh chambers.

The subjects in the NN group, as well as the NH and NS animals, had access to wire mesh chambers through a four inch segment of plexiglas tubing attached to their home cage. The chambers were roughly semicircular in shape with the same diameter as the running wheels (14"). The NN subjects were otherwise undisturbed except for weekly recordings.

Isolation controls and group housed animals.

The isolation controls (NNI) were individually housed in cages similar to those of all other subjects (46 x 24 x 20 cm, opaque polypropylene) and received no treatments other than BP and HR measurements. The group housed subjects (NNG) were rehoused after baseline measurements with three animals in the cage.

All stress and exercise treatments lasted six weeks. Body weight and rectal temperatures were taken the day after treatments were terminated.

Results

Running Wheel Activity

Continuous exercise groups.

As previously found, all animals in the freely available voluntary exercise conditions (groups CH and CS) used the wheels extensively. Running wheel activity during the course of the experiment is shown in Figure 5. There was a positive linear trend in running over weeks ($P(F = 16.23) < .01$, $df = 1,55$), with no significant difference in the distances run between these two groups. Overall, the handled group (CH) ran an average of 48.83 ± 1.88 km/wk, and the threat of shock (CS) subjects ran 45.51 ± 6.58 km/wk.

Running rates, determined by similar methods to that in Study I, had a mean of 18.6 ± 3.4 meters/min. The distance run in the hour immediately following the stress treatments was not significantly different from the hourly average during that day in neither the threat of shock nor handled groups. The average amount of running during this hour was 62% less than the amount observed in Study I.

Limited exercise groups.

The subjects in the limited exercise groups (IS and PS) ran when presented the opportunity to do so. Figure 6 shows the mean distances run in these two groups over time. As in the continuous exercise groups, there was a significant linear increase over time by trend analysis in running over weeks ($P(F = 9.92) < .01$, $df = 1,55$), with no significant difference in the distances run between the IS and PS

groups. A highly significant quadratic trend (increase followed by decrease in both groups) was also found ($P(F = 23.88) < .01$, $df = 1,55$). Overall, the IS group ran an average of 2.37 $\pm$ 0.45 km/wk and the PS group 2.48 $\pm$ 0.45 km per week.

Overall Analysis of Variance

To compare the results of this experiment to Study I, a two-way (exercise and stress) ANOVA was performed on results from the CH, CS, NH, and NS groups. For BP, there was an overall effect of the stressors to increase BP over time (stress x time: $P(F = 3.01) = .05$, $df = 5,23$), but no such effect of exercise or interaction. For HR, there was a significant effect of exercise to alter HR over time ($P(F = 4.69) < .01$, $df = 5,23$), but no significant effect of stress over time or an interaction.

Description of Additional Statistical Analyses.

To characterize the BP and HR response over time, trend analyses were performed for BP and HR in each group. This type of analysis allows a characterization of the pattern of change over time. Since this analysis involved a within-subject design, the subject by time interaction for that group was used as the error term. If a significant linear component was present in a group, slopes were generated for each subject in that condition, reflecting the rate of increase in BP or HR with time. If a cubic trend was present, slopes were similarly generated as an approximation to a linear function. If no nonlinear trends were found, group comparisons were made using the slopes of the appropriate subjects. Although a statistical comparison between groups with different types of trends (i.e., linear and quadratic) is not

possible, this result implies a difference in response pattern.

Table 2 presents the mean (± S.E.M.) blood pressures and associated trends for all groups over the course of the experiment. Table 3 depicts these results for heart rate.

Baseline Blood Pressure and Heart Rate

Initial BP and HR readings (Week 0) revealed no significant differences, based on a conservative alpha level of .10, among any groups on BP or HR. Thus, all groups started from similar BP and HR values.

Effects of stressors on BP (Non-exercised Groups)

Threat of shock vs. handling.

Figure 7 presents the time course of the effects of threat of shock (NS), handling (NH), and no stress (NN) on BP.

To determine whether BP and HR increased because of threat of shock or handling, the above three groups were compared. Analysis revealed a significant positive cubic trend in the NS group ($P(F = 8.87) < .01$, df = 1,25). BP tended to increase, decrease slightly, then increase again. A positive linear trend was found in the NH group ($P(F = 4.89) < .05$, df = 1,25). No significant linear or nonlinear trend was apparent in the NN group. No significant differences in slopes were found among these groups.

Isolation effect.

To determine if social isolation was associated with an increase in BP, the groups NNI and NNG were compared. Figure 8 presents the time course on BP of the group housed and isolated animals. The NNI animals

had a significant negative linear trend ($P(F = 7.85) < .05$), $df = 1,6$), while the group housed subjects showed a positive quadratic trend ($P(F = 10.39) < .05$, $df = 1,6$), due to a decrease over time followed by an increase at the last measurement.

Effects of Stressors on HR (Non-exercised Groups)

Threat of shock vs. handling.

Figure 9 presents the time course of the effects of threat of shock (NS), handling (NH), no stress (NN) on HR. Analyses revealed significant positive linear (NH: $P(F = 15.04) < .001$, $df = 1,25$; NS: $P(F = 4.64) < .05$, $df = 1,25$) and negative (increase followed by decrease) quadratic (NH: $P(F = 11.58) < .01$, $df = 1,25$; NS: $P(F = 6.53) < .05$, $df = 1,25$) trends over time in both the NH and NS groups, and a significant positive linear trend in the NN group ($P(F = 17.58) < .01$, $df = 1,25$). No differences among these groups were found when slopes were compared.

Isolation effect.

Figure 10 presents the time course of HR on the socially isolated and group housed controls. Both of these groups showed a significant positive linear trend (NNG: $P(F = 24.52) < .01$, $df = 1,6$; NNI: $P(F = 38.04) < .01$, $df = 1,6$). When the slopes of these groups were compared, there was a tendency for the NNI group to have greater slopes than the NNG group ($P(t = 1.86) < .10$, $df = 5$)

Effects of Exercise on BP: Continuous exercise.

To determine if freely available voluntary exercise would attenuate stress-induced increases in BP, the groups CH, CS, NH, and NS were compared. The continuous exercise and handled group (CH) showed a significant positive linear trend over time ($P(F = 5.04) < .05$, $df =$

1,25). The CS group showed no linear or non-linear trend. The slopes of the CS and CH groups were not significantly different.

Since there were no significant differences between threat of shock and handling treatments for either the exercised or non-exercised animals, these groups were combined for simplification. Figure 11 presents the time course of BP changes in the continuous exercise (CH + CS) and no exercise (NH + NS) groups. Both of these combined groups, regardless whether able to exercise voluntarily, showed similar linear and positive increases over time (CH + CS: $P(F = 5.68) < .05$, $df = 1,55$; NH + NS: $P(F = 4.08) < .05$, $df = 1,55$). The mean ($\pm$S.E.M.) slope for the exercise groups was 3.19 ± 1.17 and for the non-exercised groups 3.21 ± 1.43.

Limited exercise groups.

Trend analyses on the IS and PS groups showed a significant positive linear trend in the immediate exercise (IS) group ($P(F = 7.01) < .05$, $df = 1,25$) and a significant positive cubic component in the prior exercise (PS) group ($P(F = 8.84) < .01$, $df = 1,25$). A direct comparison of slopes revealed that these groups did not differ from each other.

In summary, voluntary exercise did not attenuate increases in BP in any of the experimental groups. BP did increase over time in all stress groups, but not in the unmanipulated control groups (NNI, NNG).

Effects of Exercise on HR: Continuous exercise.

Both the CH and CS groups showed significant negative (increases followed by decreases) quadratic trends over time (CH: $P(F = 22.94) < .01$, $df = 1,25$; CS: $P(F = 75.12) < .01$, $df = 1,25$), but no linear

increase. When these continuous exercise groups were combined, there was an overall significant positive quadratic trend ($P(F = 79.36) < .01$, $df = 1,55$) and no linear trend over time. In comparison, the nonexercised groups (NH + NS) showed both a significant linear increase and a quadratic trend over time (Linear: $P(F = 20.53 < .01$, $df = 1,55$: quadratic: $P(F = 12.80) < .01$, $df = 1,55$). Figure 12 presents the time course of this overall effect of continuous exercise on HR (N=12 per group).

Correlations were also computed weekly between the cumulative distance run and the change in HR from baseline for the CH and CS animals combined. This correlation reached significance after the third week ($r(11) = .58$, $P < .05$), and remained correlated at or beyond this significance level for all remaining weeks.

Limited exercise.

Trend analyses revealed a significant positive linear trend in the PS group ($P(F = 13.60) < .01$, $df = 1,25$), but failed to attain significance in the IS animals ($P(F = 3.40) < .10$, $df = 1,25$). The slopes of these two groups were not significantly different from each other.

The heart rates of the animals allowed limited exercise were not significantly different from the non-exercised subjects, and were significantly higher than in the animals with exercise continuously available (Newman-Keuls: $p < .05$).

Effect of Wire Mesh Chambers on BP

The effect of having another area which could be entered from the home cage was addressed by comparing the NN group to the NNI group. As noted, the NN group had no significant linear or nonlinear trend, while

BP's in the NNI group tended to decline. The slopes of these groups were not significantly different from each other.

Effect of Wire Mesh Chambers on HR

Both the NN and NNI group had a significant positive linear trend (see above). The corresponding slopes were not significantly different from each other.

Analyses of Weights and Temperatures

The subjects allowed continuous exercise (CH, CS) had significantly lower body weights than those not allowed exercise (NH, NS) ($P(F = 18.66) < .01$, $df = 1, 22$). The means ($\pm$ S.E.M.) of the exercised subjects was 361 $\pm$ 3.41 grams, and for the non-exercised rats 406 $\pm$ 4.54 g. No other comparisons among groups were significant.

There were no significant differences among any of the experimental conditions in rectal temperatures. The grand mean for rectal temperature was 33.22 $\pm$ 1.48°C.

Awake BP and HR

When blood pressure was taken after the termination of all treatments when the subjects were awake, rather than anesthetized, results were similar to those reported for ether. There was a tendency, nonsignificant, for exercise to attenuate HR ($F(1,23) = 3.02$, $p < .10$), but no effect in attenuating BP. Also, no differences were found among the non-exercised stressed animals (NH, NS, and NN) on BP or HR. As reported for anesthetized heart rates, the IS and PS groups did not differ on HR, but for BP the IS group had higher pressures ($P(t = 3.02 < .05$, $df = 5$). The means $\pm$ S.E.M. of all awake BP and HR's are presented in Table 4.

There was a significant correlation between BP's recorded while awake and under ether ($r = 0.49$, $N=42$, $p < .01$). This correlation was not significant for HR, however ($r = 0.17$, N.S.). These recordings made in awake animals were compared to those made ten days before (Week 5) under ether. Both blood pressures ($P(F = 6.31) < .05$, $df = 1,41$) and heart rates ($P(F = 8.09) < .01$, $df = 1,41$) were higher under ether anesthesia. The mean ($\pm$S.E.M.) blood pressure and heart rate under ether over all groups (except NNI and NNG) was 168 $\pm$ 4.23 mmHg and 462 $\pm$ 4.52 bpm. While conscious, the means were 153 $\pm$ 4.06 mmHg and 433 $\pm$ 8.83 bpm for BP and HR, respectively.

Discussion

The hypotheses tested in this experiment were that daily threat of shock stress would result in increases in BP and HR over time; daily "handling" would also cause increases in BP and HR; freely available voluntary exercise would attenuate increases in BP and HR due to daily stress; and limited exercise immediately after stress would be more effective in attenuating increases in BP and HR due to stress than a limited amount of exercise before stress application.

BP seems to have increased as a function of the daily stresses of threat of shock and "handling", although the increase in the threat (NS) group was nonlinear. Thus it appears that daily handling and transfer to a different environment can increase BP as much, if not more, as this "handling" plus threat of shock. In fact, in an acute experiment, McCarty and Kopin (1978a) found that the increase in mean arterial pressure (MAP) after transfering the subjects' to a shock chamber was not significantly different in animals that had experienced footshock in

that environment and in those that had not. In normotensive adult rats, handling and transfer to another room has resulted in increases in levels of plasma C (e.g., Iams, McMurtry, & Wexler, 1979; Fortier, 1959). Handling and placement into a test chamber has resulted in increases in plasma corticosterone levels larger than those found immediately after shock delivery (Friedman et al., 1967), as well as increases in plasma epinepherine and norepinepherine levels from baseline in normotensive rats. (Kvetnansky, Sun, Lake, Thoa, Torda, & Kopin, 1978). SHR rats have also exhibited consistent large increases in MAP and HR after being lifted by the tail for 30 sec (Kudo, Sokabe, & Kawashima, 1983). Thus, previous work has shown that the dramatic effects on physiology of daily handling and transfer to a different environment need not be surprising. As in Study I, exposure to odors of previously stressed rats could also have contributed to the increases in BP and HR in these groups.

Although the isolated controls showed a different trend over time than that of the group housed subjects, inspection of Figure 8 suggests no meaningful effect of isolation on BP.

In the non-exercised subjects, both the threat of shock and "handling" resulted in increases in HR over time, followed by a slight decrease (see Fig.10). A linear increase in the nonstressed (NN) subjects, and a nearly significant effect of social isolation, suggests that these increases in HR may have been partially due to housing conditions. During most weeks, the non-exercised and "handled" group (NH) actually had higher heart rates than their threat of shock counterparts. The "handled" animals were not placed into an environment previously associated with shock, but into an environment in which

conspecifics may have recently shocked. Thus, the putative stressors involved in the "handling" treatment were social isolation, daily handling and transfer to a different environment, and possibly exposure to odors of shocked conspecifics. Since the threat of shock group also presumably received all but the latter stimulation, the HR and BP response in the NH group suggests that this proposed olfactory stimulation may have caused a cardiovascular response in the animals indistinguishable from the response to threat of shock. Such a suggestion must remain speculative, however, since the effect of handling and transfer to a different environment was confounded with a possible effect of this olfactory stimulation. This confounding variable could be eliminated by placing the animals in the "handled" group into chambers in which conspecifics had never received shock.

The linear increases in HR in the nonstressed and unmanipulated control groups (NN, NNI, NNG) were unexpected. Social isolation may have contributed somewhat to the increases in the NN and NNI groups, since a nearly significant effect of isolation was found when the slopes of the NNI and NNG subjects were compared, albeit on the basis of a small number of subjects. The third study sought to determine the reliability of the apparent effect of isolation on HR. However, HR of the group housed animals also increased over time. Presumably, the increase in HR in this group reflects some uncontrolled variable which also may have contributed to the increase in HR found in the other groups. This general increase in HR in all groups was corroborated when data from Study I and II were compared. In the first study, continuous exercise lowered HR in the stressed animals. In this study, the stressed animals showed an attenuated increase in HR due to continuous

exercise, not a decrease. That this increase over time in all groups was due to some nonspecific effect was also supported since none of the nonexercised groups had increases in HR significantly greater than those of the maturational controls.

The most likely explanation for this uncontrolled effect involves the effect of repeated anesthetization on heart rate. Ether anesthetization is a known neuroendocrine stressor and has been shown to acutely increase HR, and to lower mean arterial blood pressure, (Smith & Hutchins, 1980) in both adult SHR and WKY rats. By comparing recordings made while subjects were awake to those made several days before, under ether, the expected increase in HR when anesthetized was found. Thus recordings made under ether may be relatively more stressful, in terms of the demand placed on the animal, than during conscious recordings. It was assumed that although ether may act as a stressor in this way, the effect of daily 50 min treatments would outweigh any systematic effect of the five to ten minutes of exposure per week to ether. However, it is possible that repeated exposure to ether may have acted as a chronic stressor, resulting in a progressive increase in HR, especially in those animals that were otherwise unhandled. In these animals, the stress of anesthetization would not be outweighed by the relatively more prominent daily stressors of placement into the operant chambers. According to this explanation, the progressive increase in HR in the three control groups would be due to repeated ether exposure, and the increases in the other groups due mostly to the daily treatments. That these increases in HR may have been due to some aspect of the measurement procedures was indirectly supported in that awake HR's showed a weak positive correlation to anesthetized heart rates, while BP

showed a much stronger relationship when measured under the two conditions. If the use of ether was affecting the control groups more than the other groups, this overall correlation would be expected to be lower, since the HR of these groups would be differentially affected. This correlation would be assumed to be high if there was no such differential effect. However, one could also predict that if any effect of repeated anesthetization would be found, a physiologic adaptation resulting in a lowering of HR would occur.

One reason for using ether was to reduce measurement variability, in both within-animal and between subjects Compared to variability during recordings on conscious animals, within-subject variability for BP after Week 5 showed a decrease of 17% (nonsignificant), on the average, when ether was used. Within subject variability for HR could not be estimated since only one value was scored for each animal. Between subject variability in HR was reduced by half, and was unaffected for BP. Use of ether anesthesia for BP and HR recordings, therefore, can modestly but not significantly reduce variability of recordings.

In this experiment, freely available voluntary exercise did not attenuate stress-induced increases in BP. Although the threat of shock and continuous exercise group did not show an overall increase in BP over time, when the two continuous exercise groups were combined the rate of BP increase was nearly identical to that seen in the non-exercised animals. BP values in conscious subjects corroborated these results. Thus, the effect of voluntary exercise in attenuating stress-induced HT in Study I may reflect a Type I error. Alternatively, from inspection of the time course of the effect of exercise on HR (Fig. 12),

the effects of exercise are manifested later than those of stress. Perhaps a longer extension of the treatments, as in Study I, would have resulted in a significant effect. In the study by Cox et al. (1985), significant differences between the stress only and stress plus forced-swim groups became apparent after 10 weeks of treatments, with a definite trend in this direction noticeable as early as four weeks. In this study, however, no such trends were apparent during the six weeks of treatments. Also, for both BP and HR, the animals experiencing threat of shock did not show the greatest rate of increase. This treatment may not have been as stressful as intended. The nonexercised "handled" group also did not show a consistent increase in BP or HR. If BP had risen at a faster rate in these groups, an effect of exercise may have been easier to distinguish.

However, a clear effect of continuous exercise was found on HR. As shown in Figure 12, heart rates of animals subjected either to daily "handling" or threat of shock showed linear increases in HR, leveling at Week 2. If running wheels were freely available, the initial increase in HR was followed by a gradual but consistent decline in HR to levels close to baseline values. This gradual decline in HR, when averaged with the increases in HR seen in the non-exercised animals, may explain the lack of a significant overall effect of stress to increase HR over time. The observed decline in HR in the exercising rats roughly parallelled the increase in running over time (see Figure 6), which peaked at Week 4. Correlations between the cumulative distance run and the changes in HR from baseline allowed a demonstration of how much running is necessary to result in an exercise training effect on HR. This correlation became significant at Week 3, when the animals in the

continuous exercise groups were running about 25 miles/week. This distance may be the minimum amount necessary to be reflected in a training effect in this strain.

Body weight is reduced by physical training (Tipton, 1984). That the animals allowed to freely exercise did indeed show an exercise training effect was corroborated in that their body weight at the conclusion of treatments was lower than that of the nonexercised subjects. Similarly, the conclusion that the amount of exercise engaged in by the animals allowed limited exercise (IS, PS) was not sufficient to induce a training effect was confirmed since the body weights of these animals were significantly higher than the rats allowed continuous exercise, and not different from the nonexercised subjects.

No effect of "having another area to enter from the home cage" was found for either BP or HR. Thus, the attenuating effect of continuous exercise on HR appears to be specific to physical exercise and not just to the ability to enter another area.

Conclusions

The following conclusions can be drawn from this experiment. Daily "handling" and threat of shock appeared to be similarly effective stressors, since similar increases over time were found. Some uncontrolled variable or variables such as repeated anesthetization may have contributed, in part, to these increases. Social isolation may also have been a factor. Regardless of the nature of these increases, continuous exercise attenuated increases in HR, but not BP. The effect of exercise on BP may not have been apparent due to the inconsistent rise in BP in the nonexercised stressed animals, masking any

differential effects of exercise. Continuous exercise clearly lowered heart rate from its peak values. That this attenuation of HR was in fact due to physical exercise was corroborated in that the exercised animals had lower body weights and having another area to enter from the home cage had no demonstrable effect. The importance of the temporal relation between exercise and stress treatment could not be evaluated since the time alloted to run in these limited exercise groups was not sufficient to induce an exercise training effect.

STUDY III

In Study II, there was some indication that social isolation may in itself tend to increase HR. The third study was conducted to clarify the effects of social isolation on HR and BP.

Method

Ten adult male BHR's, 10 weeks of age, served as subjects. These animals, from two litters of SHR male-WKY females, were housed five to a cage until measurements began. No more than three subjects from a given litter were assigned to a given experimental condition.

Baseline blood pressures and heart rates were taken under light ether anesthesia on two afternoons, two days apart (see Method, Study II). Two baseline measurements were taken to provide stability of the results. After the second baseline measurements were made, the animals were randomly assigned to either the social isolation or group-housed condition, with the precondition that there were no significant differences ($p < .10$) between the groups on any measurements taken. Five animals were individually housed, and the remaining animals rehoused together. One week later, BP and HR recordings were made. This period of time has been shown to be sufficient to show an effect of isolation on HR in rats (Gardiner & Bennett, 1977). Change scores were calculated based on the difference between the recordings made after treatments and the mean of the two baseline measurements.

Results

Table 5 presents the means ± S.E.M. for both BP and HR for both groups during the course of the experiment. An alpha level of .05 was used for all comparisons.

Blood Pressure

BP in both the group-housed (G) and isolated (I) subjects did not change significantly when the two baseline measurements were taken in either group, or overall. Overall means for the first reading was 159 ± 5.52 mm Hg, and for the second baseline reading 156 ± 7.30. Change scores were calculated for each animal by subtracting the mean of the two baseline values from the BP values after treatments. There was no effect of isolation on BP (t test), as the mean change in BP for the I group was -3.2 ± 9.72 and for the G group -1.0 mm Hg. There was no significant increase in BP from the mean baseline values in either group, or using results from both groups combined.

Heart Rate

Heart rates of the two baseline measurements also were not significantly different in either group, or for both groups combined. The overall mean for the first reading was 412 ± 9.2, and for the second baseline measurement 413 ± 12.0 bpm. Change scores were computed as above. There was a significant effect of isolation to increase HR ($P(t = 2.31) < .05$, df=8), as shown by analysis of change scores. The isolated animals had a mean change score of 83 ± 13.19, while the group housed controls increased by 28 ± 19.79 bpm.

There was a significant increase in HR from the mean baseline recording to the third measurement a week later in the I group ($P(t = 6.31) < .01$, $df=4$), but not in the G group.

Discussion

The major result of this study was the observation of the effect of social isolation on increasing HR, but not BP. This finding corroborated the trend (noted in the second study) of isolation to increase HR under these conditions, and previous findings using awake normotensive rats (Gardiner & Bennett, 1977). As found in the second study, there was no effect of repeated measurements to increase BP, but there was an overall effect of repeated recordings to increase HR. This result, found after only one week of isolation, supports the hypothesis that HR measurement while under ether may function as a stressor and contribute to increases in HR, especially in animals that are otherwise unhandled. Although an unanesthetized control group was not included, it can be speculated that the important aspect of the repeated measurements was that of repeated anesthetization. Since the increase in HR was considerably greater in the I group than the G group, this increase in the I group could also be attributed to the effects of isolation and not repeated HR measurement. This study, however, does not rule out a possible interaction between housing condition and the effects of repeated measurements, since these effects were confounded.

GENERAL DISCUSSION

The major findings of this project were as follows: Blood pressure (BP) increased due to shock stress and threat of shock, as well as with daily handling and transfer to the shock chambers, in borderline hypertensive rats (BHR). Heart rate (HR) also increased due to these stressors, and to social isolation.

Freely available voluntary running wheel exercise did not consistently block these increases in BP due to stress, implying that the effect of exercise interacts with the type of stressor given. Voluntary exercise blocked increases in BP only in the "handled" condition in Study I, but not increases due to other stressors. However, voluntary exercise did reliably attenuate increases in HR due to shock stress, threat of shock, and "handling" in both studies I and II.

It seems that the amount of shock stress necessary to reliably increase BP and HR in these animals is close to the levels used in Study I, i.e., approximately 100-300 one sec shocks per session (Lawler, Barker, Hubbard, Cox, & Randall, 1984; Lawler et al., 1981). If a higher density of shock had been used in the NS group in the second experiment, BP may have shown a greater and more consistent increase over time and the effects of exercise may have been more discernible.

The use of several types of putative stressors, differing in quality and intensity, suggests the use of a parametric approach in the interpretation of the results. In other words, there is a systematic relationship between the intensity of the stressor and the magnitude of the stress response, as measured by the degree of increase in BP and HR.

However, an assumption of this view is that different types of stressors, such as "handling" and shock stress, are placed on the same continuum. There is no compelling reason why this should be true (Natelson et al., 1981), though the approach has some heuristic value. It seems reasonable that increasing intensities of a given stressor would result in corresponding increases in the magnitude of the stress response. However, the results of this project indicate that when different types of stressors are evaluated in this fashion, the relative intensity of a stressor, reflected by the magnitude of the elicited stress response, is not intuitively obvious. This was most clearly seen in the first and second studies where apparently daily handling and transfer to a different environment had at least as great an effect on BP and HR as handling, transfer, and anticipation of shock. Also, the demonstrated effect of social isolation to increase HR is generally not considered in the design of experiments. Especially when variables are being investigated which are affected by the stress response, the potential effect of supposedly benign stressors should not be overlooked.

These results underscore the importance of the use of controls for the effects of handling, transfer, and social isolation in experimental designs. In this context, the separation of the effects of several different types of stressors provides an opportunity to test the effect of each of these treatments on the stress response, and to determine the relative magnitude of these putative stressors in a more empirical fashion.

Any generalization about how seemingly benign treatments may act as stressors must be limited, since all the present work was conducted on a

particular (inbred) strain of laboratory rat. In fact, the BHR has been shown to be more reactive in cardiovascular responses to chronic stress than their Wistar-Kyoto progenitor strain (Lawler et al., 1984). This animal model may therefore be appropriate for an understanding of the responses to stress and exercise in a cardiovascularly reactive population, such as borderline hypertensives (Julius & Schork, 1971) or Type A individuals (Glass, 1977).

Heart rate appeared to be more sensitive to environmental changes than BP, as shown in both an effect of social isolation on HR, and an increase in all groups in HR (possibly due to repeated ether stress in Study II). These effects were not seen in BP. Also, when follow up measures were taken four weeks after termination of treatments in the first study, HR had returned to near baseline values, while BP remained elevated. These results could be interpreted as implying that either HR is relatively more sensitive than BP or shows a more rapid adaptation to environmental changes than BP, which may relate to differences in the physiologic control of HR and BP. The alteration of BP setpoints is generally considered as posing a greater risk to the health of the organism. When the circulatory system is stressed, homeostatic mechanisms may work to more closely regulate BP than HR. Also, there are more interactive control mechanisms to hold BP constant than to keep HR at a set level (Berne & Levy, 1977). Thus, BP may be less likely to vary from a certain setpoint at the expense of fluctuations in HR. However, one must be cautious in attributing any changes in HR to emotional or motivational states, since HR is not a particularly useful index of these states (Obrist, 1981).

In these studies, BP was recorded by several methods. BP was measured directly via the femoral artery, and indirectly by tail cuff recordings made in awake and anesthetized rats. Unfortunately, there exists no method of BP recording which provides a "true" measure of basal BP. Among these methods, direct measurements probably give the most accurate appraisal of basal BP. However, limitations of this method include the impact of a catheter inside the animal, the stress associated with surgery, and the difficulty in making repeated measurements in the same animal. For studies of chronic stress, this latter limitation is a serious one.

There are also problems associated with the indirect tail cuff method of recording. The animal must be warmed and restrained, and random tail and body movements can affect the output of the pulse sensing device (Borkowski & Quinn, 1983). Recordings made in this manner in some ways reflects BP and HR responsiveness rather than basal values. Also, several readings must be taken while the animal is quiet, making for a relatively time consuming method. However, BP's recorded indirectly have been shown to have a high positive correlation, on the order of 0.80 to 0.90, to BP measured directly (Bunag, 1973).

Indirect BP can also be measured under ether anesthesia, as in Study II. This method eliminates problems associated with the effect of heating and restraining the animal, and with body movements. This method also allows rapid determinations of BP and HR. The major problem with this method, however, is that the process of exposure to ether fumes may be a potent stressor, especially because of its effect on HR. Ether has been shown to elevate levels of both plasma cathecholamines (CA: Borkowski & Quinn, 1983; Buhler, DaPrada, Haefely, & Picotti, 1978)

and corticosterone (C: Seggie & Brown, 1975) in normotensive Sprague-Dawley and WKY rats. However, restraint associated with indirect recordings (Chiueh & Kopin, 1978), but not ether anesthesia (Borkovski & Quinn, 1983), has been shown to elevate plasma E levels in SHR rats. Thus, both ether and restraint may have exacerbating effects on plasma CA levels.

The use of ether anesthesia in BP and HR measurement has been ostensibly validated in that BP recordings made under ether had a high positive (0.86) correlation with direct pressures, and that in the present work, BP recorded under ether also had a significant, but lower, positive correlation (0.49) with recordings made while awake. However, the correlation between awake and anesthetized animals on HR was low, and ether may have acted as a chronic stressor on the HR of subjects in the second experiment, particularly in the control groups. The variability of measurement was somewhat lower under ether anesthesia. Use of a different anesthetic which would be injected rather than inhaled could possibly eliminate the putative stressor of ether administration, but would not solve the problem of getting an unbiased measure of resting BP and HR.

Voluntary running wheel exercise was shown to be an effective method to establish exercise training, since the expected training-induced bradycardia and weight loss were found in animals allowed to exercise freely (Scheuer & Tipton, 1977). The distances run by the animals, after a three week period of adaptation to the wheels, ranged from 22 to 46 miles per week. These distances are comparable with previous reports of how much normotensive rats of comparable ages will run in a running wheel, which ranged from 15.3 to 45.7 mi/wk

(Hellhammer, Rea, Bell, & Belkein, 1984; Goodrick, 1980; Ring, Dupuch, & Creed, 1967; Smith & Dugal, 1965).

The limited exercise treatments, involving 90 minutes of running wheel availability in Study II, were not sufficient to induce these training effects. Three hours of voluntary running in normotensive rats (Starzec et al., 1983) and two hours of swimming in the BHR (Cox et al., 1985) appeared to be sufficient to manifest a training effect. Perhaps making the wheels available for a longer period (2 to 3 hours) would have resulted in a training effect in these animals.

Voluntary exercise is an effective paradigm for investigating the effects of concurrent stress on the cardiovascular system. One reason may be that during voluntary exercise, the animal can regulate its physiologic state. In contrast, during forced exercise, the degree of exertion is externally imposed. During forced exercise, the demands placed on the heart may be more severe. It is possible that excessively high levels of venous return may be reached, which may acutely depress rather than enhance the pumping capacity of the ventricles, as reflected in a decrease in stroke volume and myocardial contractility (Berne & Levy, 1977). In contrast, voluntary exercise would presumably not involve excessive demands on the cardiac tissue.

The intensity of exercise can be estimated by the rate of running (Scheuer & Tipton, 1977). In these experiments, running rate was determined by estimating the meters/min during running in several animals. Forced treadmill running typically involves between 60-80% of the maximal exercise intensity, while voluntary exercise in the present studies involved an estimated 40-60% of the animal's maximal exercise

intensity. These estimates thus support the possibility that forced exercise may place demands on the cardiovascular system over and above those which would be made voluntarily.

In addition, chronic forced exercise may result in increases in basal C plasma levels (Kant et al., 1983), while voluntary exercise does not (Starzec et al., 1983). This potential difference in C levels may play a significant role in the modulation of the effects of E and NE on the heart and vasculature.

The failure to replicate the effect of voluntary exercise on stress-induced increases in BP in Study II could mean that the effect found in the first study was either a sampling, or a procedural error.

The fact that both sexes were used in Study I, while only males were run in Study II, should not have an influence on the results, since there was no significant effects of gender in Study I. The age of the subjects, however, may have made a difference. Lawler et al. (1984) found a difference in how much BP rose due to the same chronic stress treatment depending on the age of the BHR subjects. They stated that "there may be a critical period during which environmental influences have their greatest effect" (p.104). The subjects in Study I were 16 weeks at the start of treatments, and those in the second study were 10 weeks of age. Combining these age differences and results from Lawler's laboratory (Lawler et al., 1984; Lawler et al., 1981) with the present results, it is possible that such a critical period may occur somewhere between the ages of 11 and 17 weeks. Before or after this age, the stressors of shock-shock conflict, threat of shock, and "handling" (Study II) may not result in as great an increase in BP. Also, the

cardiovascular changes associated with exercise training are more extensive in older subjects (Tipton, 1984). With these considerations, animals in this type of study ideally should be between 15 and 17 weeks of age.

The shock densities used in Study I and II also may have made a significant impact on the results. In Study I, the effect of exercise to attenuate BP was significant only in animals who had received relatively high density shock for eight days, followed by zero density shock. In Study II, the effect of exercise was not found in groups who had been exposed to either relatively moderate density shock for one week followed by low density shock (CS, NS), or in subjects who had received no shocks (CH, NH). The effects of exercise on BP may be most easily seen in animals who had experienced a large change in shock density, as in the "handled" group of Study I, where a high shock density was followed by a zero shock density. Other factors, such as social isolation and possible exposure to odors from previously stressed rats, were relatively constant for animals in both studies. If this hypothesis is true, then if a higher density of shock had been used during the first week in Study II, perhaps BP would have increased in the NS group at a faster rate, providing a greater likelihood of detecting a difference between the exercised and non-exercised stressed groups.

The rise in BP in the first study was similar for all three stressors of signalled shock, unsignalled shock, and "handling", but measurements were taken only before and after treatments. In the second study, recordings were made weekly, allowing a more detailed description of the course of changes in BP. Although the BP of the subjects

receiving daily threat of shock and no exercise (NS group) did have a significant cubic trend, the net change in BP from baseline recordings to the final week was less (12.4 ± 13.7) than the similar change in BP in the comparable group (HNE) in Study I (46.4 ± 9.3), although the method of BP measurement differed. This latter group never received footshock after the first week, while the NS group continued to receive five shocks per week. Thus, it was surprising that the NS group did not show increases in BP of a similar if not greater magnitude than the HNE group. It is possible that exposure to the first week of random shock buffered the subjects from the threat of occasional shock that followed. The sharpest increase in BP did occur over this first week of treatments. Alternatively, perhaps in the HNE group in Study I, BP continue to stay elevated not due to threat of shock, but due to daily handling and transfer and/or exposure to odors of previously stressed rats.

There is no obvious basis for concluding that the effect of exercise on stress-induced HT was in fact reliable. Taking into account the work of other investigators, particularily that of Cox et al. (1985), one could tentatively conclude that the exercise effect on stress-induced HT is real, but small. An additional replication, with conditions as similar as possible to that in the "handled-group" in Study I, and an increased sample size, would be advised.

The effect of voluntary exercise to attenuate increases in HR due to stress, however, were robust and consistent in both Study I and II. The most likely explanation is the development of training-induced

bradycardia, even when concurrent with stress treatments. Therefore, exercise training can be a valid protocol to block development of tachycardia and its associated risks, regardless of the effects of concurrent stressors, at least in this animal model.

In Study I, exercise resulted in a bradycardia independent of stress. In Study II, exercise protected against the increase in HR due to stress, but did not lower HR below baseline levels. This is a potentially important distinction in terms of mechanism, since if exercise results in TIB independent of stress, the effects of stress and exercise may be acting via different mechanisms. Since the results were not consistent in this regard, determination of the actual mechanism involved in TIB awaits further clarification.

One problem apparently not addressed in the literature, however, is how exercise, which involves acute sympathetic activation (e.g., Cohen & Obrist, 1975), can after repeated bouts possibly result in lowered basal sympathetic tone and HR (Scheuer & Tipton, 1977), while a typical stressor, also causing short-term activity of the SNS as previously described, can cause increases in basal sympathetic tone (e.g., Kvetnansky et al., 1979), HR, and BP (e.g., Buchholtz et al., 1981) when presented chronically. Three general explanations are possible, based on different hemodynamic, neural, or humoral adaptations to chronic exercise and stress.

The explanation based on hemodynamic differences during the response to stress and exercise has been outlined as the local autoregulation (LA) hypothesis. The central argument for this hypothesis is that the hemodynamic adjustments made during the response

to a "classic" stressor and those made during physical exercise are different. During exercise, TPR is lowered more than during stress (Langer et al., 1979), due to the increased blood flow required by the active muscle. Thus, one event leading to chronically increased TPR, local autoregulatory constriction in skeletal muscle beds, would not occur. The sum of the evidence, however, points against support for the LA hypothesis, mainly due to the results from the pilot experiment and those of Djojosugito et al. (1968). These results suggest that the presumed autoregulatory constriction ends as soon as the stressor is terminated, removing the substrate that exercise following stress is presumed to act on. However, in the present context, an adequate evaluation of the LA hypothesis was not possible because no hemodynamic measurements were made, such as an estimation of TPR in various vascular beds immediately after stress, and the limited exercise protocol was not sufficient for an exercise training effect. It remains quite possible that exercise <u>during</u> stress would block the hypertensive effects of stress by halting the postulated vasoconstriction in skeletal muscle. Technical difficulties regarding the stressing of animals while they are running did not permit this idea to be tested.

A more likely explanation is based on differential changes in autonomic nervous system responsiveness or its sequelae after chronic exercise or stress. Although repeated stress may not increase sympathetic responsiveness (Kvetnansky et al., 1979), concurrent exercise training may act by attenuating the effects of SNS activation (Cox et al., 1985). As mentioned, there are several ways this effect could be transduced, including directly dampening the SNS response to stress, lowering the ratio of stress-induced sympathetic/parasympathetic

cardiac stimulation, which may also play a role in development of intrinsic HR changes (Lewis et al., 1980), and/or blocking SNS responsiveness in renal beds.

A final somewhat speculative explanation for this apparent paradox relies on observed differences in the synthesis and utilization of adrenal hormones after chronic stress and exercise treatments. Repeated immobilization stress in rats has been associated with increased rates of adrenal CA synthesis (Kvetnansky & Mikulaj, 1970; Kvetnansky, Weise, & Kopin, 1970). In contrast, exercise training has been shown to decrease the rate of CA synthesis (Matlina, 1984; Tipton, 1984; Peronnet, Nadeau, deChamplain, Magrassi, & Chatrand, 1981) and increase the utilization of adrenal CA (Harri & Kuusela, 1981), thus reducing circulating CA levels at rest (Scheuer & Tipton, 1977). The glucocorticoids may play a modulating role in these effects. After prolonged exercise, adrenal CA synthesis was restored with exogenous glucocorticoid treatment, but in non-exercised rats, this treatment did not affect rates of CA synthesis (Matlina, 1984). Basal plasma C levels do not increase after voluntary exercise in rats (Starzec et al., 1983), and resting blood cortisol levels may decrease after physical training in man (Tipton, 1984), while basal plasma C levels do increase after chronic stress treatments (Kant et al., 1983). Thus, it can be postulated that stress may increase steroid production which would normally be used in ensuing physical activity (Connell, Cooper, & Redfearn, 1958). If exercise is then engaged in, an increase in the utilization of glucocorticoids would occur, causing decreased circulating levels of these hormones and an inhibition of adrenal CA synthesis. In the absence of exercise, no such suppression of adrenal

CA synthesis would occur, either on an acute or chronic basis. This suppression of CA synthesis after exercise, but not after treatment with other stressors, may be a mechanism to protect the body from excessive CA action (Matlina, 1984). This effect could account for the effects of exercise on attenuating stress-induced increases in HR and/or BP, since C (Kalimi, 1982) and CA (Scheuer & Tipton, 1977) receptors are known to be found in cardiac tissue. The validation of one or more of these hypotheses could be empirically determined in later work.

In conclusion, the studies reported in this dissertation have shown that voluntary running wheel exercise can be an effective exercise treatment; that this form of exercise is effective in blocking increases in HR due to chronic stress in the BHR; and that this particular strain is quite sensitive to relatively mild environmental perturbations. It is hoped that the logical extensions of this research can be carried out in future work.

APPENDIX

PILOT EXPERIMENT

Aim

The aim of this preliminary study was to document changes in BP due to aspects of the stress procedures employed in these experiments, and to see if BP would remain elevated after removal from the shock chamber as predicted by the LA hypothesis.

Method

Subjects

Three rats were used as subjects in this study. Subject 1 was a male SHR age 16 months. Subject 2 was an SHR female five months old. The third rat was a Sprague-Dawley (SD) male four months of age. These animals were used since they were from a subject population no longer involved in the breeding colony. No adult BHR's were available.

Procedure

Femoral artery cannulation.

Subjects were anesthetized with sodium pentathol (Nembutal, 2 mg/kg, i.p.) and an incision made where the right hind limb joins the torso. The femoral artery was isolated and tied with surgical (4.0) silk distal to the area of cannulation. The artery was clamped, and a small cut made below this point. The flanged cannula (PE-10), filled with heparinized (10%) saline, was then inserted into the artery for a distance of about 1.5 cm. The clamp was then released and the cannula tied firmly in place both around the artery and to adipose tissue near the artery. The cannula was then reflected dorsally and superiorly, and led subcutaneously under the skin to emerge at the back of the neck on the midline. At the point of exit, a 5 cm length of Silastic tubing was

attached to the cannula. The heparinized saline in the cannula was then withdrawn by a syringe and replaced with 100% heparin to avoid blood clotting during recovery from surgery. The Silastic was then wrapped around a forceps, sealed with an amphenol connector, and placed underneath the skin. The wound was closed with sutures. The animal was then returned to its home cage and allowed to recover for 24 hours. All surgery took place between 10 and 12 AM. The surgical procedure lasted approximately 90 minutes.

Blood pressure recordings.

The following day, the animal was transferred from the colony room to the laboratory and lightly anesthetized with ether. The sutures were removed and the cannula exposed. The heparin was withdrawn by a syringe and replaced with a 10% heparinized saline solution. Another 20 cm section of PE-10 tubing was then attached to the Silastic tubing. The first 20 cm of the exposed cannula was then fitted inside of a much thicker tubing (PE-190) to reduce the possibility of the animal biting its cannula. This tubing was affixed with adhesive tape to the cannula to avoid displacement. The end of the cannula was then connected to a 30 gauge needle and syringe. The rat was then returned to its home cage in the animal colony room and closely monitored during recovery from anesthesia. The needle was attached to a Statham pressure transducer, in turn connected to a Grass (Model 79D) polygraph. Recordings were considered valid only if a stable pulse pressure was seen at a sensitivity level close to or the same as that used for the recordings.

Procedure.

All animals had undergone exposure to five consecutive daily sessions

of 50 min of one sec, 0.4 mA scrambled inescapable shock delivered through the grid floor of a standard operant chamber (Gerbrands Model D) within the previous week. The shock was delivered on a random basis, with a median intershock interval of four minutes. Thus, during each session 10 to 15 shocks were delivered.

Data collection began at least one hour after the animal had recovered from anesthesia, as noted by normal movement. Direct blood pressure (DBP) was recorded every five minutes for twenty minutes while the animal was relatively quiet in its home cage (time -20 to 0 min). The animal and equipment were then transferred to the test room and DBP recorded while in its cage (time +2 min). After three minutes, the animal was then gently placed into the operant chamber where it had received the final session of random shock during the previous 24-48 hours. DBP was then recorded immediately upon placement into the chamber, and after five minutes (time +5 to +10 min). Five minutes later, a one sec shock was then delivered, and DBP recorded both immmediately and five minutes therafter (time +15 to +20 min). The animal was then removed from the chamber and placed in its home cage in the test room and another recording made (time +22). The animal was then returned to the colony room and DBP recorded every minute for the next five minutes (time +25-30 min).

Results

Although the subjects varied in terms of age, sex, and strain, the changes in DBP from baseline recordings during the procedures were quite consistent. The initial DBP values (at time -20 min) were as follows: Subject 1 (SHR male): 111 mmHg; Subject 2 (SD male): 108 mmHg; Subject 3

(SHR female): 138 mmHg. Figure 13 presents the mean changes in blood pressure during the test procedure. Little change in DBP was noted during the 20 min of baseline recordings or during transfer to the test room. A marked increase in DBP occurred upon placement into the operant chamber, and a furthur increase found immediately after the one sec shock. Pressures then quickly dropped to a level below that seen upon first entering the chamber. Immediately upon removal from the chamber, DBP returned to near baseline levels and remained in this range after return to the colony room. The increased variability seen during the final DBP measurement was probably due to variations in the behavior of the animals. One animal was fairly quiet during this time, but the others were self-grooming and/or moving about the cage. If the subject was active, DBP continued to be monitored until the animal became relatively quiet, which occurred within 10 min. In both cases, the pressures recorded at that time were within 5 mmHg of the mean baseline value.

Discussion

Blood pressure, as measured directly via the femoral artery, was found to increase from baseline readings after placement into an environment previously associated with shock and increased further immediately after shock, as expected from previous work by McCarty et al. (McCarty, Chiueh, & Kopin, 1978; McCarty & Kopin, 1978a; McCarty & Kopin, 1978b). However, the major result of this study was that blood pressure returned to near baseline values immediately after removal from the operant chamber. This agrees with BP data from borderline hypertensive men, in which BP returned to near baseline levels within

two minutes after cessation of mental arithmetic stress (Schulte & Neus, 1983). This result does not support the invocation of the LA hypothesis to explain the attenuating effect of exercise on BP in Study I. For the vasoconstriction in skeletal muscle beds to still be occurring when exercise begins, TPR, and presumably BP, should remain elevated after return to the home cage.

Previous work (Djojosugito et al., 1968) has also shown that in an in vitro preparation, the locally mediated increase in resistance lasts no longer than the increased flow. Thus, if the return of BP to baseline levels reflects a lowering of CO, it may be tentatively concluded that the component of the TPR due to these locally mediated events returns to basal levels concurrent with normalization of BP. The LA hypothesis cannot be ruled out at this juncture, however, without at least a further indirect test of the hypothesis in borderline hypertensive animals. Of course, a truly direct test of this hypothesis would require a description of structural and resistance changes in precapillary muscle beds due to stress.

One unexpected result was the relatively low blood pressure found in subject 1 (SHR male). The most likely explanation for these readings is some form of measurement error, such as the tip of the cannula actually being in the abdominal aorta, for example, which, due mostly to its greater diameter, can lower BP up to 15% of its value in the periphery (Berne & Levy, 1977). Another source of error could be an inaccurate reading due to a very small leak in the system. Regardless of the absolute value of the pressures, the changes in DBP due to the test procedures were consistent with those found in the remaining subjects, implying that the measurement system was responsive to the test procedures in this animal.

Table 1

BP and HR (mean ± S.E.M.) in the six experimental groups during the course of Study I

BLOOD PRESSURE (mm Hg)

Group	Baseline	Post-avoidance	Post-stress Treatments	Four week Follow-up
signalled shock+exercise	162± 8.1	164± 7.1	192± 8.6	185± 6.6
unsignalled shock+exercise	148± 6.8	169± 7.2	170± 8.4	177±11.5
handled+exercise	165± 5.1	178± 4.5	172±13.5	165±13.8
signalled shock+no exercise	156± 4.2	167± 7.0	181± 9.3	176±12.1
unsignalled shock+no exercise	155± 6.3	171± 4.4	195± 4.9	177±11.5
handled+no exercise	149± 6.0	162± 5.2	195±10.6	187±11.3
maturational controls	156±15.3	-	-	159±12.8

HEART RATE (bpm)

Group	Baseline	Post avoid-ance	Post-stress Treatments	Four week Follow-up
signalled shock+exercise	451±12.8	398±17.3	374±20.3	450±24.9
unsignalled shock+exercise	440±14.9	421±24.5	409±25.5	459±25.6
handled+exercise	449±25.4	403±12.6	369±19.8	438±27.2
signalled shock+no exercise	439±14.2	395±45.0	454±17.1	415±17.0
unsignalled shock+no exercise	450±35.9	425±45.1	505±12.1	457±32.2
handled+no exercise	457±28.9	436±18.0	504±10.0	445±22.6
maturational controls	441±17.4	-	-	435±18.2

Table 2

BP (Mean ± S.E.M.) for all groups in Study II
over time and type of trend detected

Group	Week 0	1	2	3	4	5	Trend
IS	136±4.8	169± 9.5	152±11.3	151± 8.4	173± 9.0	169±10.0	Linear
PS	150±4.9	170±11.5	162± 8.2	178± 6.9	134± 7.4	179±11.2	Cubic
NS	136±6.5	163±14.9	140±11.1	158± 6.7	146±11.2	150±11.2	Cubic
CS	148±9.9	155± 9.9	140±13.9	158± 9.3	139± 8.5	168±13.0	None
NH	148±4.5	128±10.2	158± 9.8	177±12.6	151± 6.8	166±11.0	Linear
CH	156±8.3	150±11.0	171± 5.3	168±11.7	168± 8.8	177±13.0	Linear
NN	138±8.5	135± 2.4	136± 8.8	133± 8.7	159± 6.5	150±12.2	None

Group	Week 0	3	6	9	Trend
NNI	153±11.4	146±16.8	127±8.9	126±10.5	Linear (negative)
NNG	156±13.0	135±19.7	111±9.9	157±10.7	Quadratic

Table 3

HR (Means ± S.E.M.) for all groups in Study II over time and type of trend detected

Group	Week 0	1	2	3	4	5	Trend
IS	430±14.4	445±12.0	468±11.9	474± 4.0	457±22.2	467± 8.8	None
PS	408±30.0	462±13.5	475± 9.9	462± 9.5	475± 8.8	477±11.2	Linear
NS	420± 9.3	457±14.3	468± 8.0	458±13.0	462±14.0	462± 6.0	Linear + Quad.
CS	415± 8.3	492± 4.0	485± 7.2	465± 6.2	462± 4.0	437± 6.3	Quadratic
NH	412±13.3	457±24.4	480±12.4	497± 9.2	467± 7.0	480±11.3	Linear + Quad
CH	417±10.5	470±12.1	463±13.1	465± 8.5	442±11.7	428±11.9	Quadratic
NN	417±18.9	468±15.9	455± 7.9	472± 9.1	483±10.9	488±10.0	Linear

Group	Week 0	3	6	9	Trend
NNI	393±26.7	463± 3.3	477±12.8	482±12.3	Linear
NNG	407± 8.8	437±18.7	447±14.5	454± 7.6	Linear

Table 4

Means (± S.E.M.) of BP and HR recorded in conscious animals after termination of treatments in Study II

Group	Blood Pressure	Heart Rate
IS	175± 6.1	458±21.2
PS	147± 5.2	452±26.6
NS	150± 9.8	420±19.8
CS	137±11.2	400±31.3
NH	158±11.3	452±16.8
CH	157±13.1	408±15.4
NN	145±14.7	450±29.0
NNI	146±12.0	440±30.6
NNG	128±13.7	440±11.5

Table 5

Means (± S.E.M.) for BP and HR
for all groups in Study III

Group (N=5)	Mean Baseline BP	Final BP	Mean Change in BP	Baseline HR	Final HR	Change in HR
Group Housed	159±8.3	151±8.7	-0.8±2.6	414±12.0	442±17.1	28±19.8
Socially Isolated	155±6.7	152±6.4	-3.2±9.7	411± 8.4	494± 8.7	83±13.2

Figure 1. Experimental design for Study I. Letters within each cell refer to the abbreviated name for that condition, and numbers within each cell refer to the number of subjects.

STRESS

EXERCISE	Predictable Signalled shock	Predictable Unsignalled shock	Handled
Freely Available Voluntary Exercise	S E (6)	U E (6)	H E (6)
No Exercise	SNE (5)	UNE (6)	HNE (6)

MATURATIONAL CONTROLS

M C (8)

Figure 2. Time course of distance run for the exercise groups in Study I. Distances, in mean miles/week (±S.E.M.) in each group, is plotted as a function of week of the experiment.

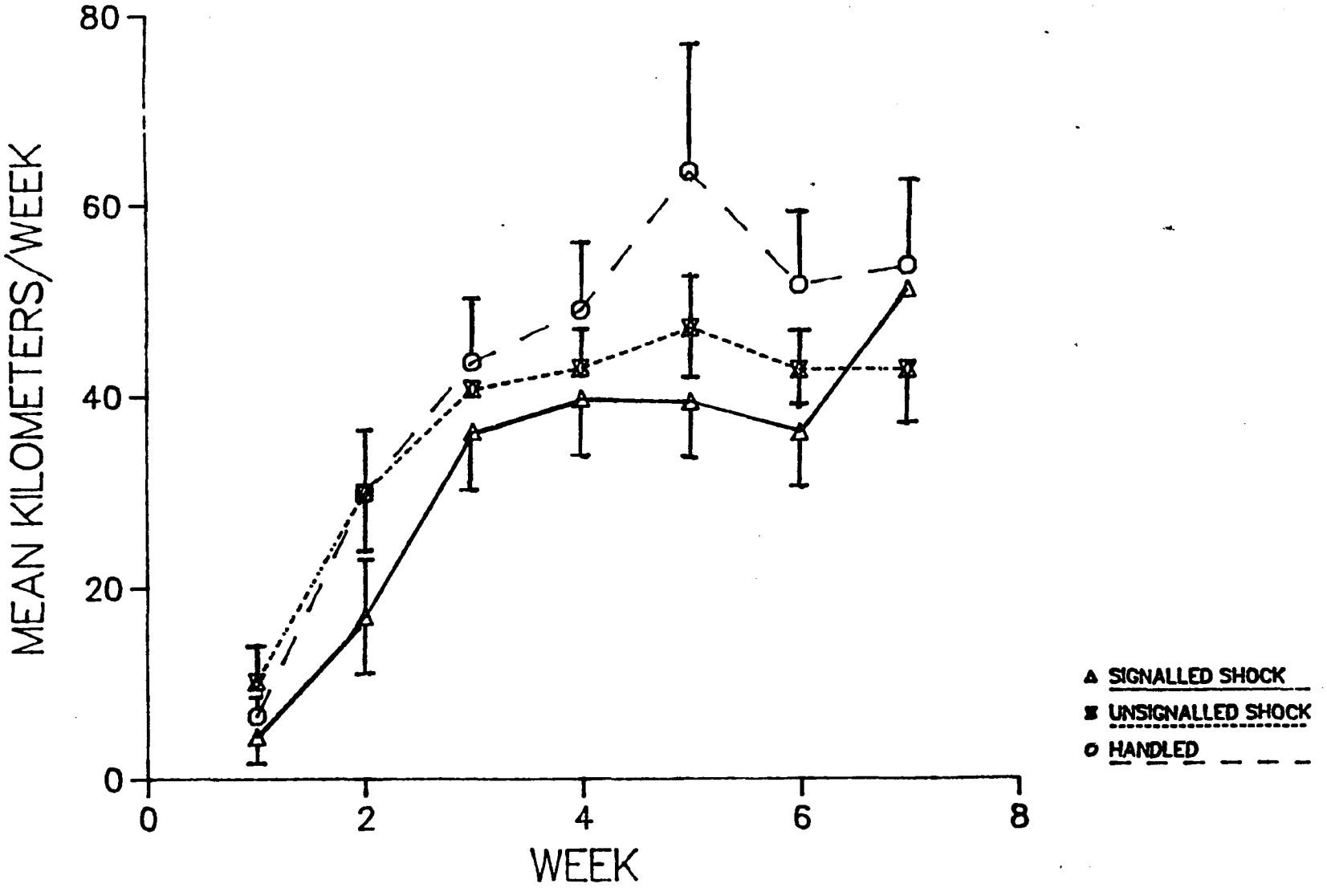
MEAN KILOMETERS/WEEK
80
60
40
20
0
0
2
4
6
8
WEEK
SIGNALLED SHOCK
UNSIGNALLED SHOCK
HANDLED

Figure 3. Plot of changes in BP (3a - 3c) and HR (3d-3f) from baseline for the six experimental groups in Study I over the course of the experiment. Initial pressures and heart rates were taken at Week 1 (age=16 weeks), post-avoidance pressures at Week 3, post-treatment values at Week 11, and measurements after the 4 week recovery period at Week 15.

Figure 3a: Changes in BP from baseline for signalled shock group. Figure 3b: Changes in BP from baseline for the unsignalled shock group. Figure 3c: Changes in BP from baseline for the handled (no shock) group.

Figure 3d: Changes in HR from baseline for the signalled shock group. Figure 3e: Changes in HR from baseline for the unsignalled shock group. Figure 3f: Changes in HR from baseline for the handled group.

Means and standard errors are presented in Table 1.

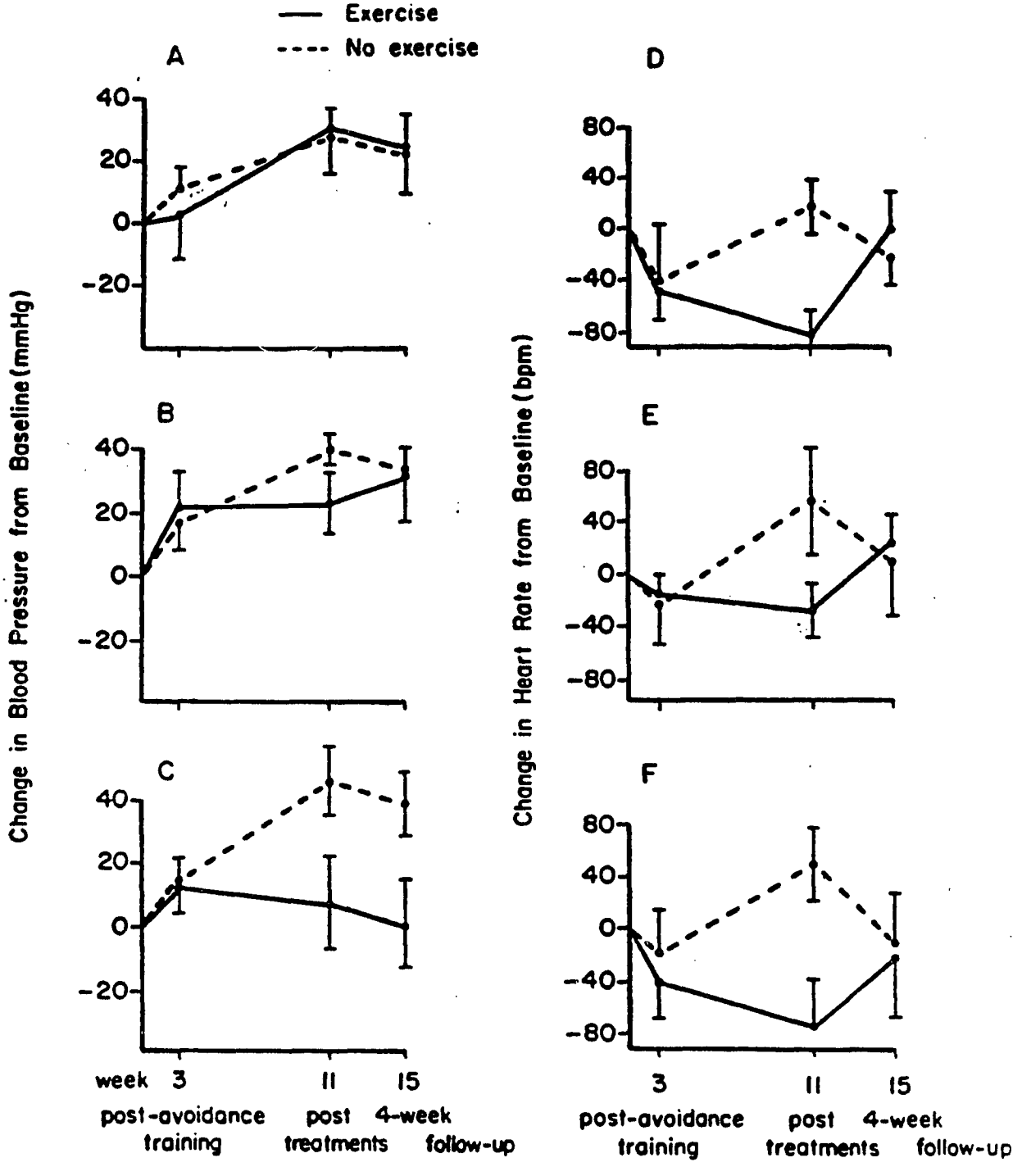
Exercise
No exercise
A
B
C
D
E
F
40
20
0
-20
80
40
0
-40
-80
Change in Blood Pressure from Baseline (mmHg)
Change in Heart Rate from Baseline (bpm)
week 3
11
15
post-avoidance training
post treatments
4-week follow-up

Figure 4. Experimental design for Study II. Letters within each cell refer to the abbreviated name for that condition, and numbers within each cell refer to the number of subjects.

STRESS

EXERCISE	Threat of Shock	Handled
Freely Available	C S (6)	C H (6)
Immediately after stress	I S (6)	—
Prior to stress	P S (6)	—
No Exercise	N S (6)	N H (6)

CONTROL GROUPS

(no stress or exercise treatment)

Socially Isolated with wire mesh chambers	N N (6)
Socially Isolated no chambers	NNI (3)
Group Housed	NNG (3)

Figure 5. Time course of distance run for the two freely available exercise groups in Study II. Distances, in mean miles/ week (±S.E.M.) in each group, are plotted as a function of week of the experiment.

MEAN KILOMETERS/WEEK
0
20
40
60
80
WEEK
0
1
2
3
4
5
6
CH
CS

Figure 6. Time course of distance run for the two limited voluntary exercise groups in Study II. Distances, in mean kilometers /week (±S.E.M.) in each group, are plotted as a function of week of the experiment.

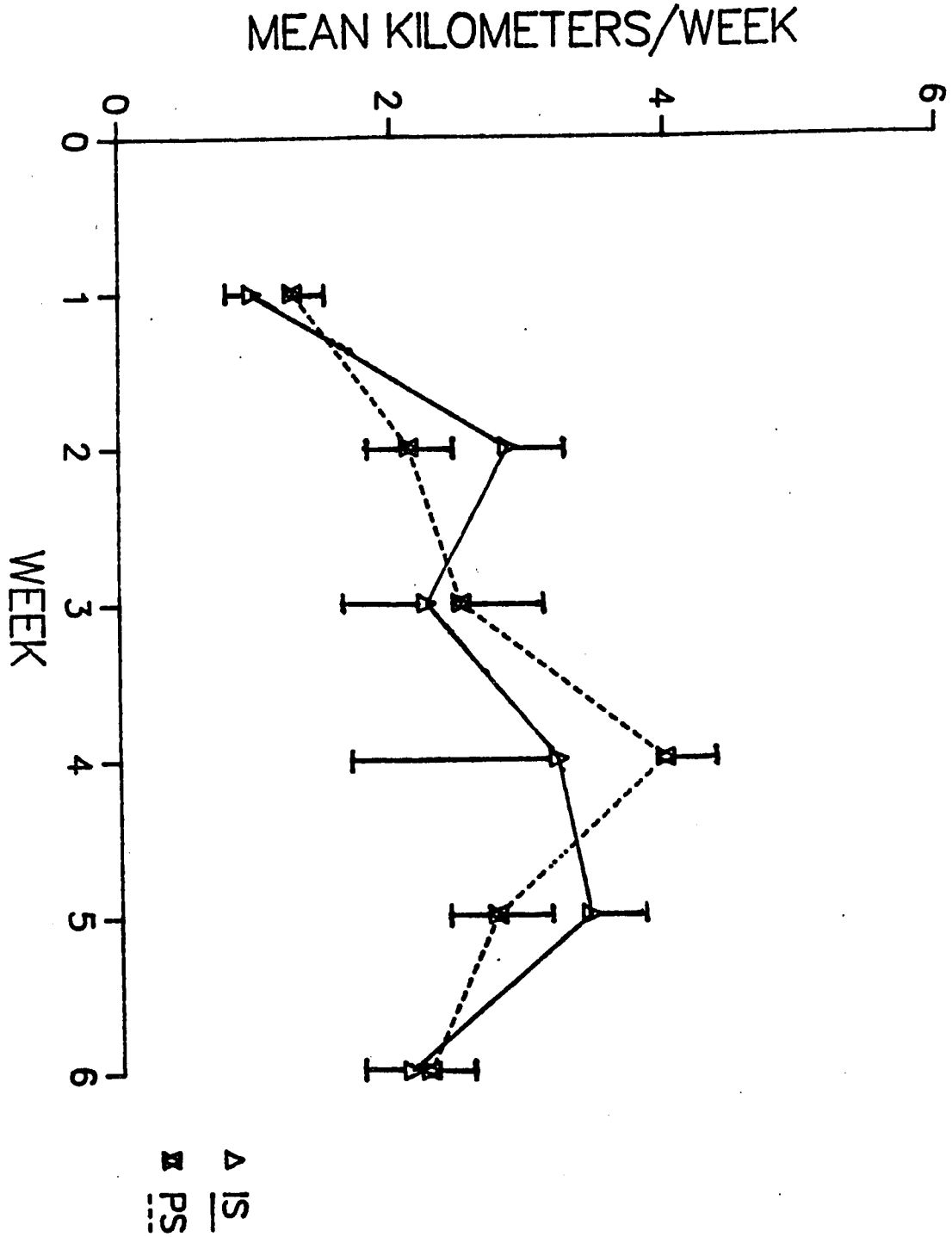
MEAN KILOMETERS/WEEK
0
2
4
6
WEEK
0
1
2
3
4
5
6
IS
PS

Figure 7. Mean BP (±S.E.M.) in the threat of shock (NS), handled (NH), and no stress (NN) groups during the course of Study II.

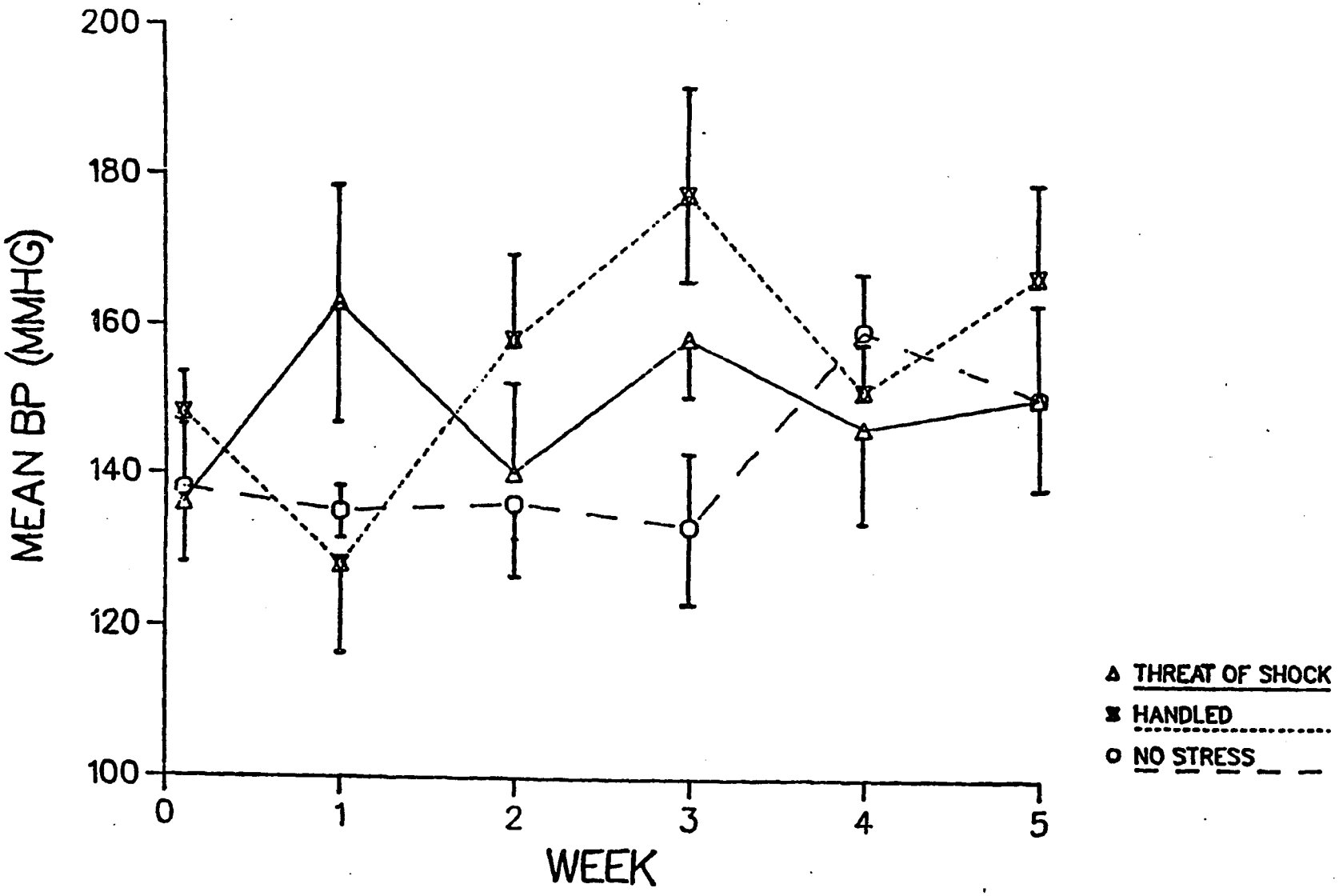
200
180
160
140
120
100
MEAN BP (MMHG)
0
1
2
3
4
5
WEEK
THREAT OF SHOCK
HANDLED
NO STRESS

Figure 8. Time course of the effects of social isolation on BP in Study II. Data points represent means ± S.E.M. for the socially isolated (NNI) and group

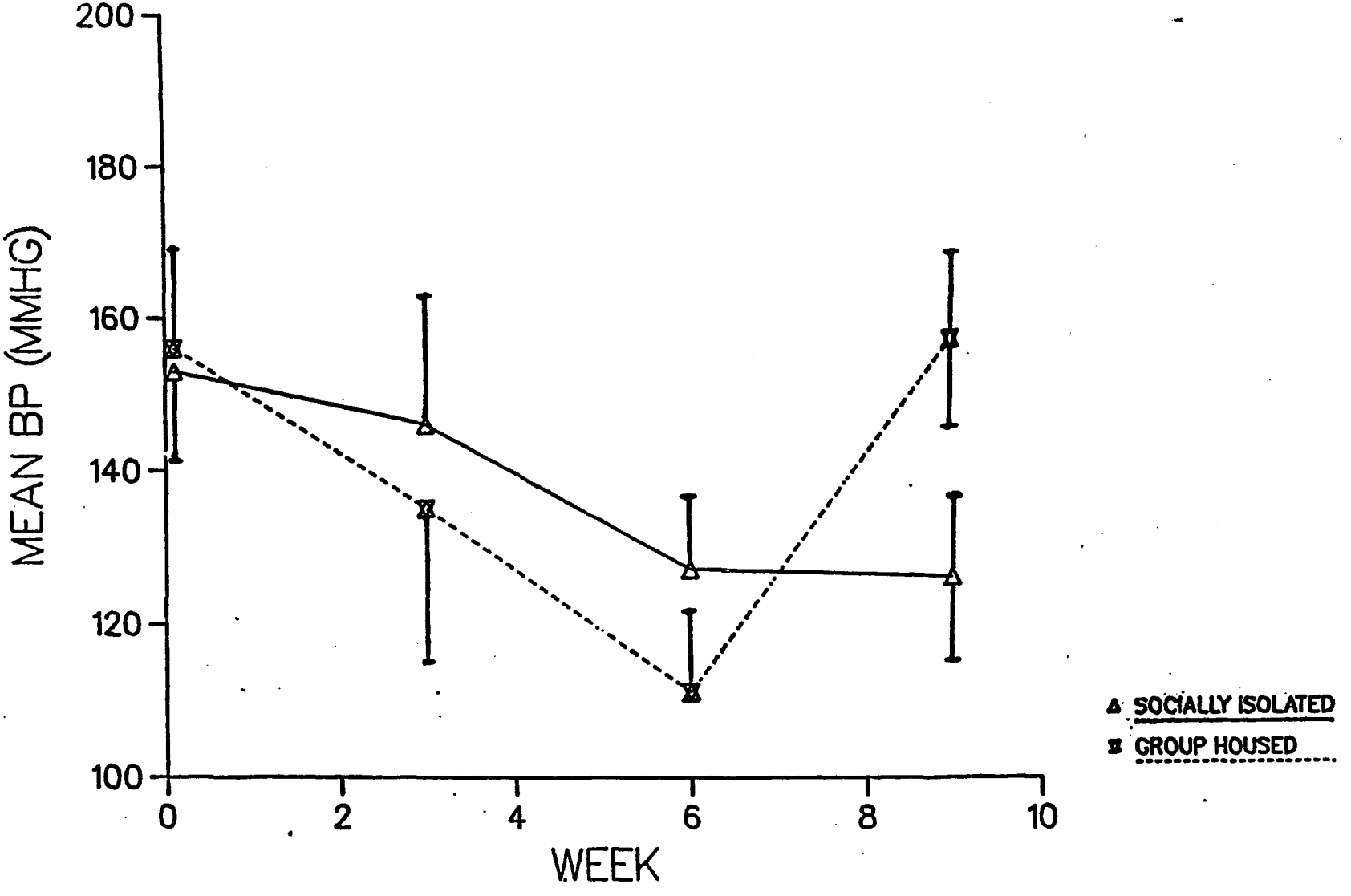
200
180
160
140
120
100
MEAN BP (MMHG)
0
2
4
6
8
10
WEEK
SOCIALLY ISOLATED
GROUP HOUSED

Figure 9. Mean HR (±S.E.M.) in the threat of shock (NS), handled (NH), and no stress (NN) groups during the course of Study II.

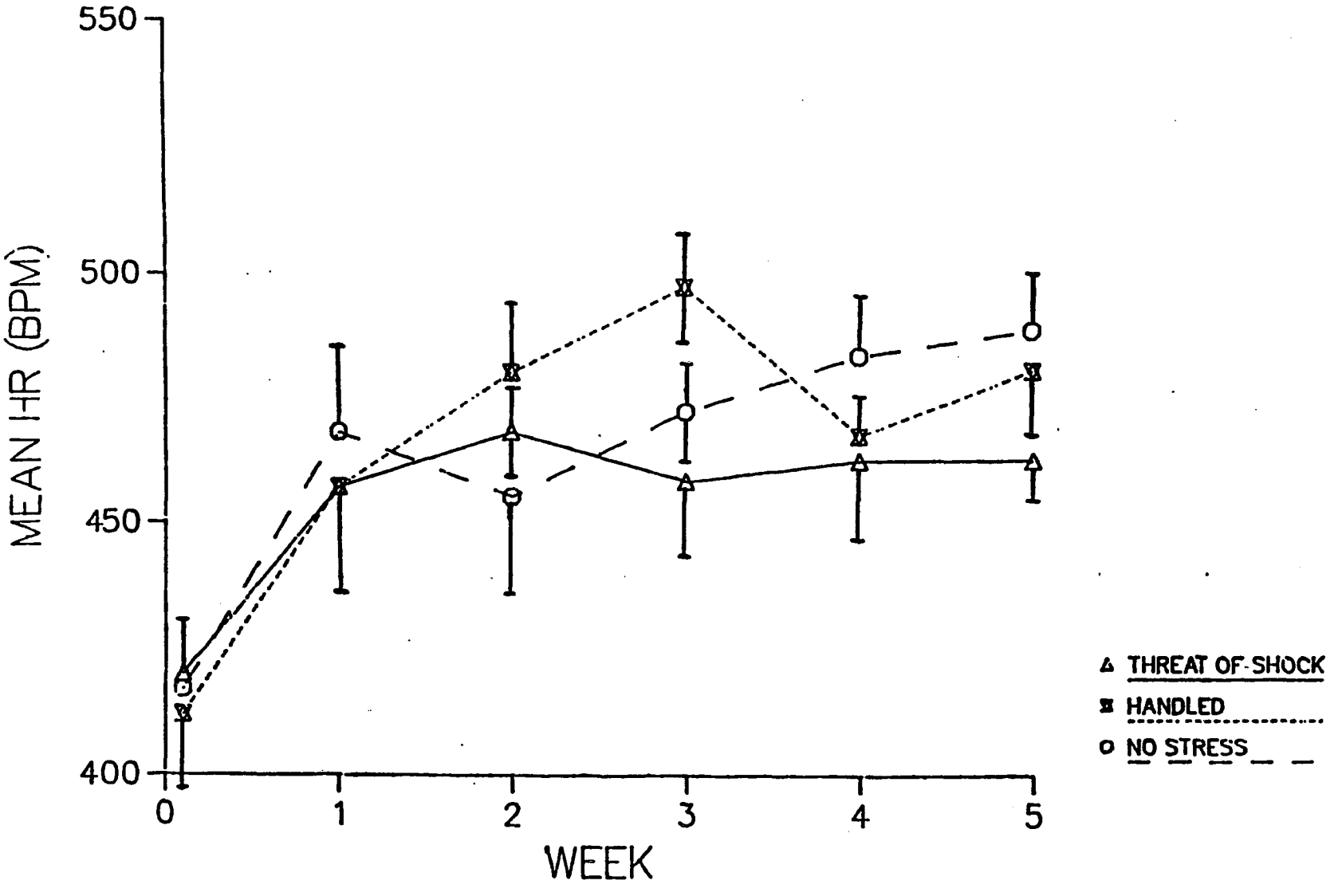
550
500
450
400
MEAN HR (BPM)
0
1
2
3
4
5
WEEK
THREAT OF-SHOCK
HANDLED
NO STRESS

Figure 10. **Time course of the effects of social isolation on HR in Study II. Data points represent means ± S.E.M. for the socially isolated (NNI) and group housed (NNG) conditions.**

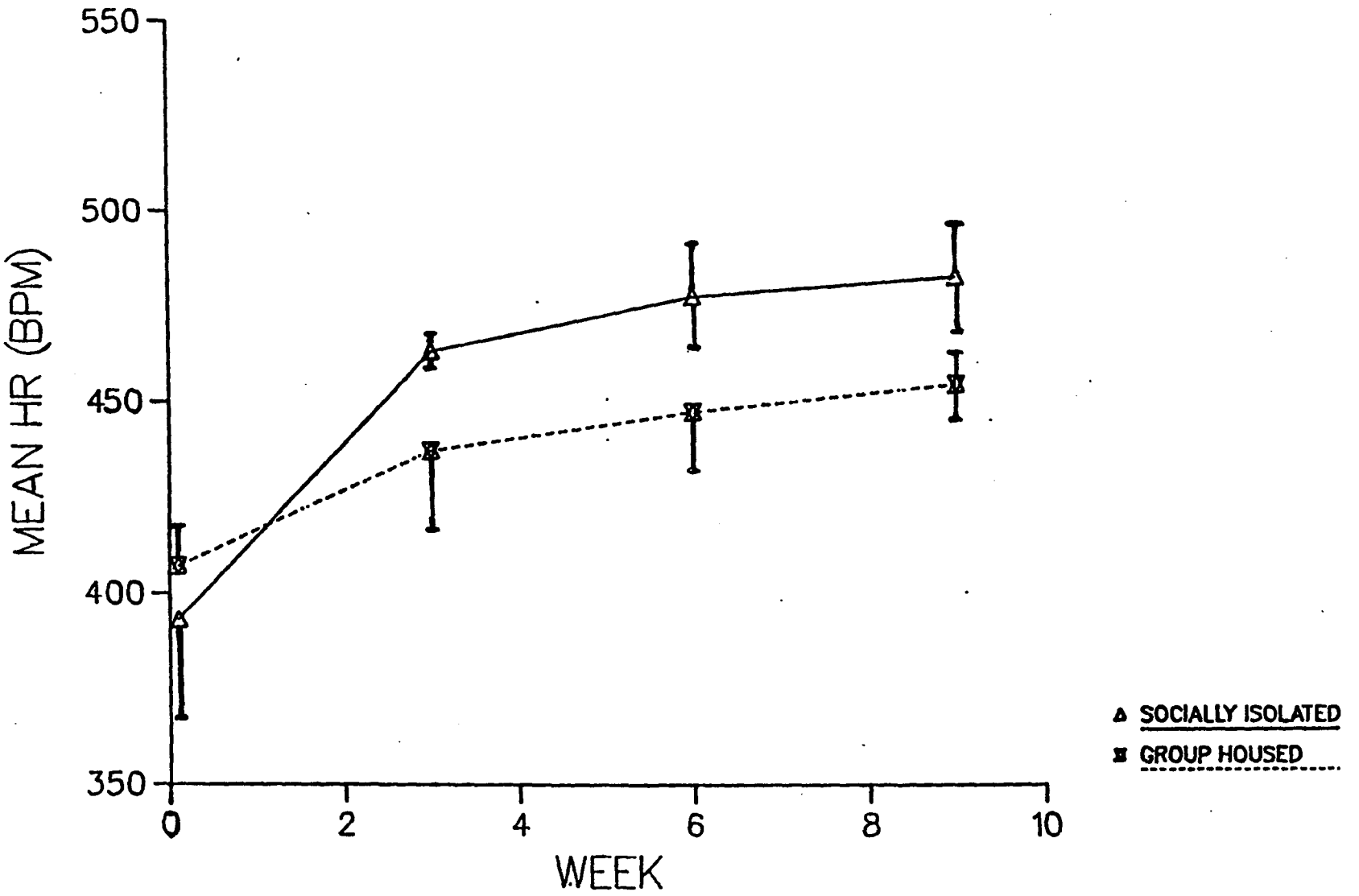
550
500
450
400
350
MEAN HR (BPM)
0
2
4
6
8
10
WEEK
SOCIALLY ISOLATED
GROUP HOUSED

Figure 11. **Time course of the overall effects of continuous exercise on mean BP in Study II. The threat of shock and handled groups were combined (N=12).**

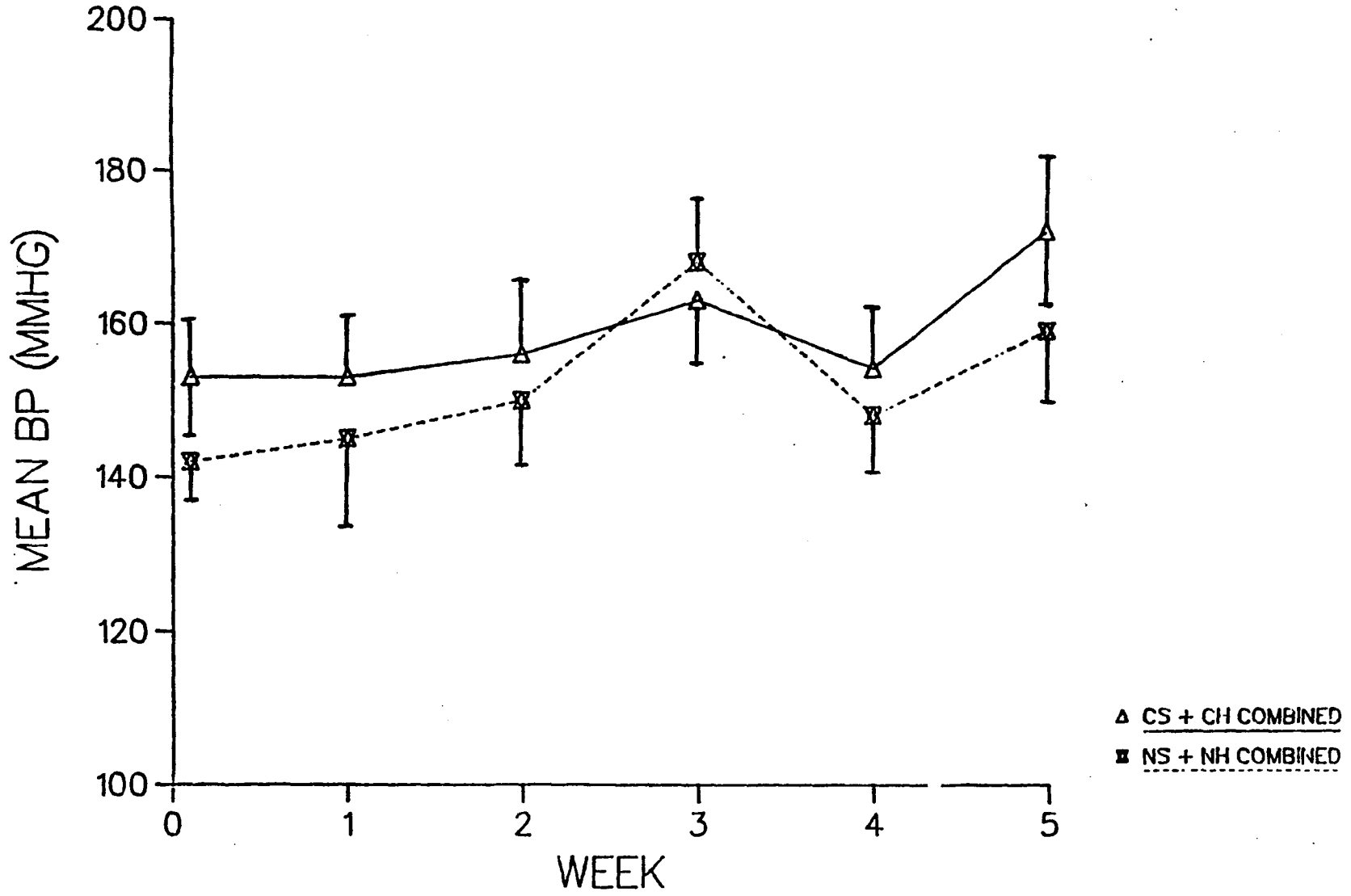
200
180
160
140
120
100
MEAN BP (MMHG)
0
1
2
3
4
5
WEEK
CS + CH COMBINED
NS + NH COMBINED

Figure 12. Time course of the overall effects of continuous exercise on mean HR in Study II. The threat of shock and handled were combined (N=12).

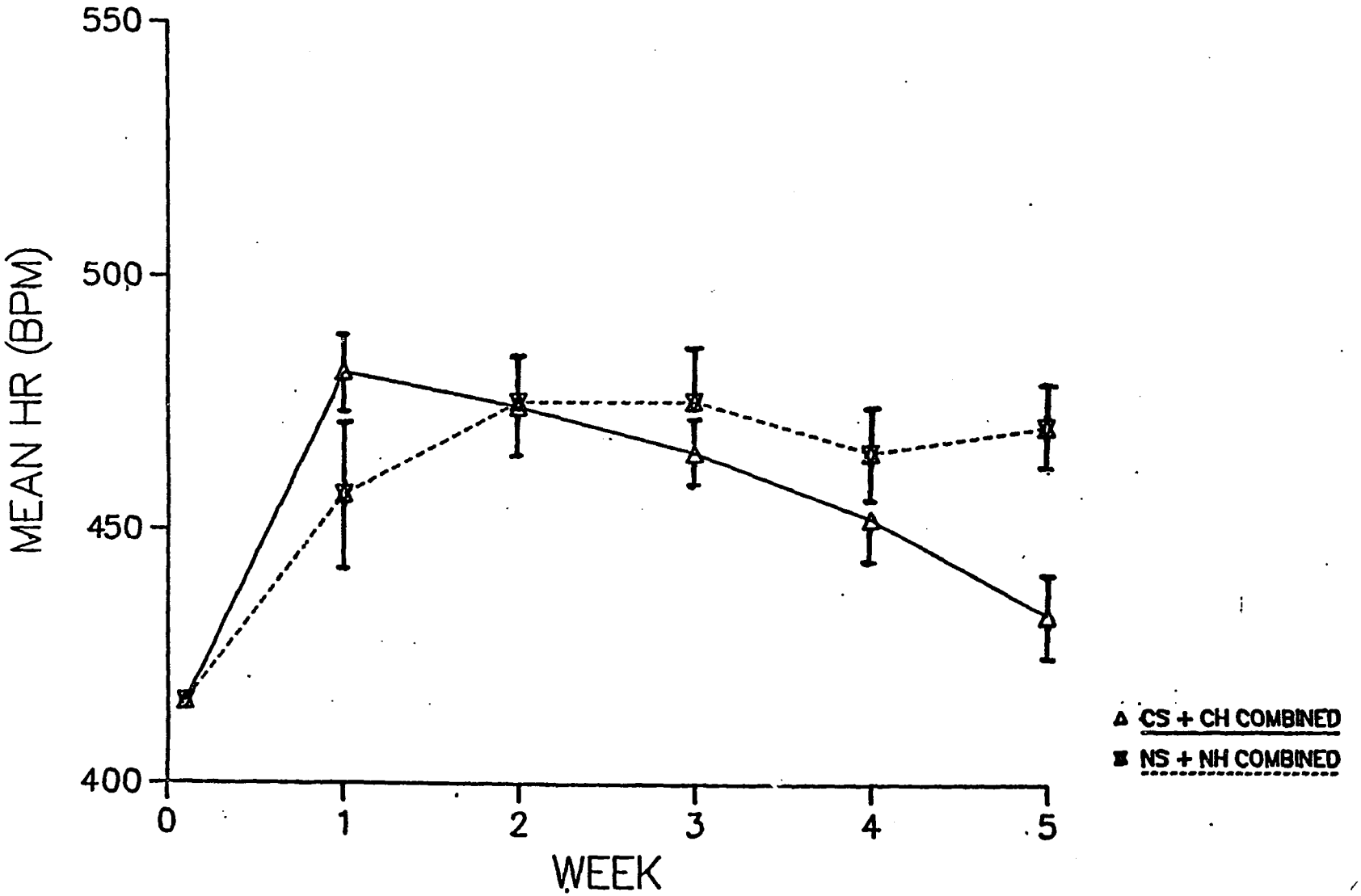
550
500
450
400
MEAN HR (BPM)
0
1
2
3
4
5
WEEK
CS + CH COMBINED
NS + NH COMBINED

Figure 13. Changes in direct BP (±S.E.M.) in the pilot experiment in three rats from baseline recordings (A), due to movement into the test room (B), placement into the operant chamber (C), application of a one-sec shock (D), removal from the chamber (E), and return to the home cage environment (F).

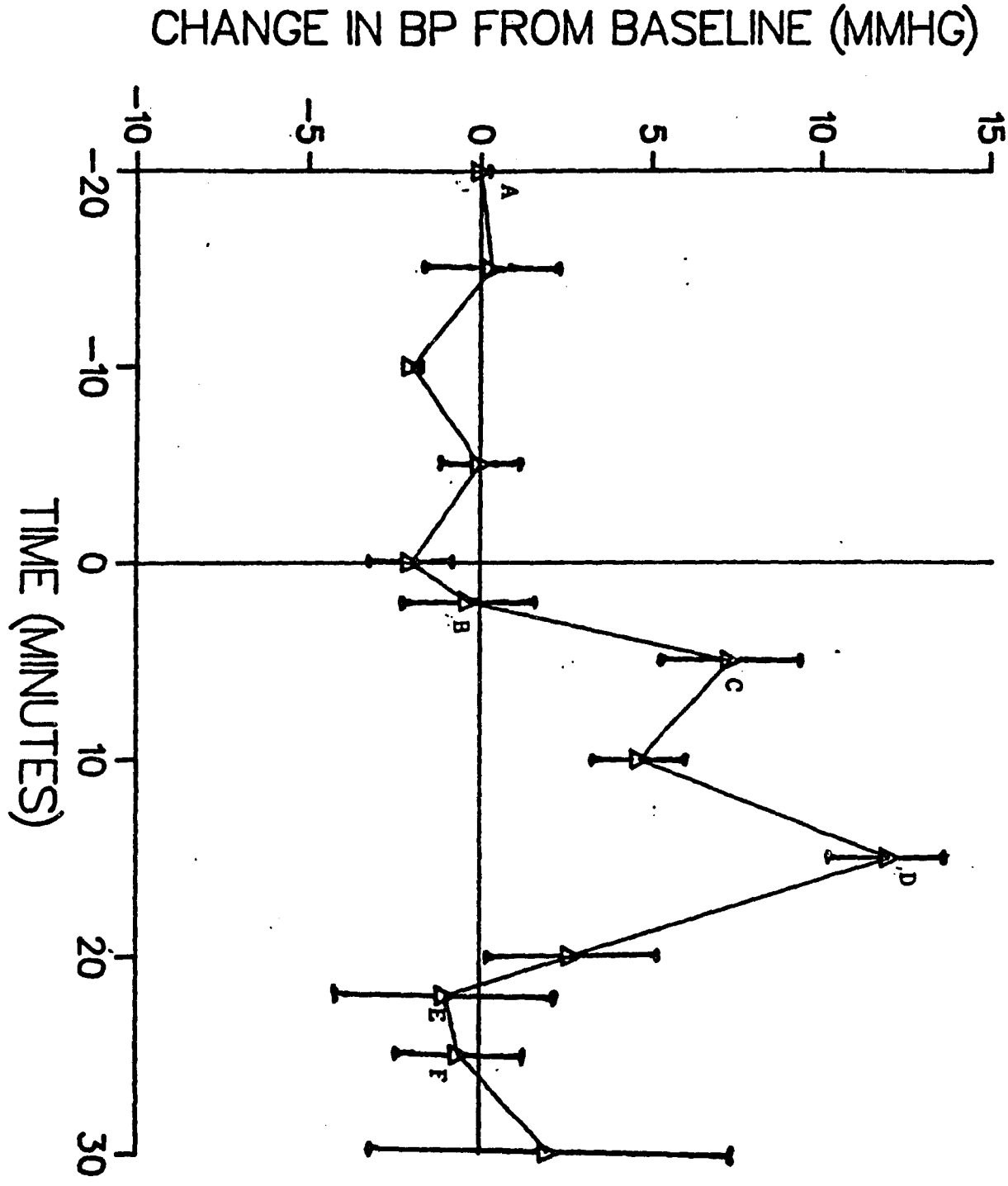
CHANGE IN BP FROM BASELINE (MMHG)
-10
-5
0
5
10
15
-20
-10
0
10
20
30
TIME (MINUTES)
A
B
C
D
E
F

Table of Abbreviations

BHR..... Borderline hypertensive rat
BP..... Blood pressure
C..... Corticosterone
CA..... Catecholamines
CH..... Continuous exercise plus "handled" group, Study II
CO..... Cardiac output
CS..... Stimulus light (Study I); Continuous exercise plus threat of shock group (Study II)
DBP..... Direct blood pressure
E..... Epinepherine
H-E..... "Handled" plus exercise group, Study I
H-NE..... "Handled" plus no exercise group, Study I
HR..... Heart rate
HT..... Hypertension
IS..... Immediate exercise plus threat of shock group, Study I
LA..... Local autoregulation hypothesis
MC..... Maturational controls, Study I
NE..... Norepinepherine
NH..... No exercise plus "handled" group, Study II
NN..... No exercise and no stress group, Study II
NNI..... No exercise, no stress, and socially isolated group, Study II
NNG..... No exercise, no stress, and group housed condition, Study II
NS..... No exercise plus threat of shock group, Study II
PS..... Exercise prior to stress plus threat of shock group, Study II
S-E..... Signalled shock plus exercise group, Study I
S-NE..... Signalled shock plus no exercise group, Study I
S.E.M..... Standard error of the mean
SHR..... Spontaneously hypertensive rat
SNS..... Sympathetic nervous system
TIB..... Training-induced bradycardia
TC..... Tachycardia
TPR..... Total peripheral resistence
U-E..... Unsignalled shock plus exercise group, Study I
U-NE..... Unsignalled shock plus no exercise group, Study I
WKY..... Wistar-Kyoto rat

REFERENCES

Abboud, F.M. The sympathetic system in hypertension. Hypertension 1982, 4 (Suppl. II), 208-225.

Abboud, F.M. Effects of sodium, angiotensin, and steroids on vascular reactivity in man. Fed. Proc. 1974, 33, 143-149.

Abrahams, V.C., Hilton, S.M., and Zbrozyna, A.W. The role of active muscle vasodilitation in the alerting stage of the defence reaction. J. Physiol. 1964, 171, 189-202.

Berne, R.M., and Levy, M.N. Cardiovascular physiology, 3rd Ed. St. Louis: CV Mosby, 1977.

Bevan, R.D. An autoradiographic and pathological study of cellular proliferation in rabbit arteries correlated with an increase in arterial pressure. Blood Vessels 1976, 13, 100-128.

Birrell, P., and Roscoe, C. Effects of intensive aerobic exercise on stress reactivity and myocardial morphology in rats. Physiol. Beh. 1978, 20, 687-692.

Blackburn, H. Non-pharmacologic treatment of hypertension. Ann. N. Y. Acad. Sci. 1978, 304, 236-242.

Blomqvist, C.G, and Saltin, B. Cardiovascular adaptations to physical training. Ann. Rev. Physiol. 1983, 45, 169-189.

Bolles, R.C. Species-specific defense reactions and avoidance learning. Psych. Rev. 1970, 77, 32-48.

Borkowski, K.R., and Quinn, P. Validation of indirect systolic blood pressure measurement in ether anaesthetised rats. J. Auton. Pharmac. 1983, 3, 157-160.

Brundin, T., and Cernigliaro, C. The effect of physical training on the sympathoadrenal response to exercise. Scand. J. Clin. Lab. Invest. 1975, 35, 525-530.

Buchholtz, R.A., Lawler, J.E., and Barker, G.F. The effects of avoidance and conflict schedules on the blood pressure and heart rate of rats. Physiol. Beh. 1981, 26, 853-863.

Buhler, H.U., DaPrada, M., Haefely, W., and Picotti, G.B. Plasma adrenaline, noradrenaline and dopamine in man and different animal species. J. Physiol. 1978, 276, 311-320.

Bunag, R.D., Takeda, K., and Riley, E. Spontaneous remission of hypertension in awake rats chronically exposed to shaker stress. Hypertension 1980, 2, 311-318.

Bunag, R.D. Validation in awake rats of a tail-cuff method for measuring systolic blood pressure. J. Appl. Physiol. 1973, 34, 279-282.

Bush, I.E. Chemical and biological factors in the activity of adrenocortical steroids. Pharmacol. Rev. 1962, 14, 317-408.

Cannon, W.B. Bodily changes in pain, hunger, fear, and rage (2nd ed.). New York: Appleton, 1915.

Cannon, W.B. The wisdom of the body. New York: W.W. Norton, 1932.

Carew, T.E., Dennis, C.A., and Covell, J.W. Left-ventricular function in exercise-induced hypertrophy. Fed. Proc. 1974, 33, 380.

Chiueh, C.C., and Kopin, I.J. Hyperresponsivity of spontaneously hypertensive rats to indirect measurement of blood pressure. Am. J. Physiol. 1978, 234, H690-H695.

Cohen, D.H., and Obrist, P.A. Interactions between behavior and the cardiovascular system. Circ. Res. 1975, 37, 693-706.

Coleman, H.N., Taylor, R.R., Pool, P.E., Whipple, G.H., Cowell, J.W., Ross Jr., J., and Braunwald, E. Congestive heart failure following chronic tachycardia. Am. Heart. J. 1971, 81, 790-798.

Connell, A.M., Cooper, J., and Redfearn, J.W. The contrasting effects of emotional tension and physical exercise on the excretion of 17-ketogenic steroids and 17-ketosteroids. Acta Endocrinol. 1958, 27, 179-194.

Convertino, V.A., Brock, P.J., Keil, L.C., Bernauer, E.M., and Greenleaf, J.E. Exercise training-induced hypervolemia: role of plasma albumin, renin, and vasopressin. J. Appl. Physiol. 1983, 43, 665-669.

Cox, R.H., Hubbard, J.W., Lawler, J.E., Sanders, B.J., and Mitchell, V.P. Exercise training attenuates stress-induced hypertension. In preparation, 1985.

Davis, J.O. Control of renin release. Hospital Practice 1974, 9, 55-65.

Dawson, C.A., and Horvath, S. Swimming in small laboratory animals. Med. Sci. Sports 1970, 2, 51-78.

Djojosugito, A.M., Folkow, B., Lisander, B., and Sparks, H. Mechanism of escape of skeletal muscle resistance vessels from the influence of sympathetic cholinergic vasodilator fiber activity. Acta Physiol. Scand. 1968, 72, 148-156.

Ekblom, B., Kilbom, A., and Soltysiak, J. Physical training, bradycardia, and the autonomic nervous system. Scand. J. Clin. Lab. Invest. 1973, 32, 251-256.

Falkner, B., Onesti, G., Angelako, E.T., Fernandes, M., and Langman, C. Cardiovascular response to mental stress in normal adolescents with hypertensive parents: Hemodynamics and mental stress in adolescents. Hypertension 1979, 1, 23-30.

Fanselow, M.S. Odors released by stressed rats produce opioid analgesia in unstressed rats. Beh. Neurosci. 1985, 99, 589-592.

Fenske, M., Fuchs, E., and Probst, B. Corticosteroid, catecholamine and glucose plasma levels in rabbits after repeated exposure to a novel environment or administration of (1-24) ACTH or insulin. Life Sci. 1982, 31, 127-132.

Folkow, B. Primary hypertension. Physiol. Rev. 1982, 62, 347-504.

Folkow, B. The fourth Volhard lecture. Cardiovascular structural adaptation: its role in the initiation and maintenance of primary hypertension. Clin. Sci. Mol. Med. 1978, 55, 3s-22s.

Folkow, B., and Neil, E. Circulation. New York: Oxford University Press, 1971.

Forsyth, R.P. Regional blood flow changes during 72-hour avoidance schedules in monkey. Science 1971, 173, 546-548.

Fortier, C. Sensitivity of the plasma free corticosteroid response to environmental change in the rat. Arch. Int. Physiol. 1958, 66, 672-677.

Friedman, R., and Iwai, J. Dietary sodium, psychic stress, and genetic predisposition to experimental hypertension. Proc. Soc. Exp. Biol. Med. 1977, 155, 449-452.

Friedman, S.B., Ader, R., Grota, L.J., and Larson, T. Plasma corticosterone response to parameters of electric shock stimulation in the rat. Psychosom. Med. 1967, 29, 323-328.

Gardiner, S.M., and Bennett, T. The effects of short-term isolation on systolic blood pressure and heart rate in rats. Med. Biol. 1977, 55, 325-329.

Gilmore, J.P. Physiology of stress. In: Stress and the heart. R.S. Eliot (Ed.). New York: Futura, 1974. Pp. 69-90.

Glass, D.C. Behavior patterns, stress, and coronary disease. Hillsdale, N.J.: Erlbaum, 1977.

Goldstein, D.S., Harris, A.H., and Brody, J.V. Sympathetic adrenergic blockade effects upon operantly conditioned blood pressure elevations in baboons. Biofeedback Self Reg. 1977, 2, 93-105.

Goodrick, C.L. Effects of long-term voluntary running wheel exercise on male and female Wistar rats. Gerontology 1980, 26, 22-23.

Groen, J.J., Hansen, B., Herrmann, J.M., Schafer, H., Schmidt, T.H., Selbmann, K.H., v.Uexkill, T.V., and Weckmann, P. Effects of experimental emotional stress and physical exercise on the circulation in hypertensive patients and control subjects. J. Psychosom. Res. 1982, 26, 141-154.

Guttman, M.C., and Benson, H. Interaction of environmental factors and systemic arterial blood pressure: a review. Medicine 1971, 50, 543-555.

Guyton, A.C., Coleman, T.G., and Granger, H.J. Circulation: Overall regulation. Ann. Rev. Physiol. 1972, 34, 13-46.

Harri, M., and Kuusela, P. Effects of the adrenergic nervous system on training-induced cardiac enlargement, and on the intrinsic rate and phenylephrine sensitivity of isolated rat atria. Can. J. Physiol. Pharmacol. 1982, 60, 1125-1130.

Hellhammer, D.H., Rea, M.A., Bell, P.M., and Belkein, L. Activity-wheel stress: effects on brain momamines and the pituitary-gonadal axis. Neuropsychobiology 1984, 11, 251-254.

Henry, J.P., Meehan, J.P., and Stephans, P.M. The use of psychosocial stimuli to induce prolonged systolic hypertension in mice. Psychosom. Med. 1967, 29, 408-432.

Henry, J.P., Stephans, P.M., and Santistaban, G.A. A model of psychosocial hypertnesion showing reversibility and progression of cardiovascular complications. Circ. Res. 1975, 36, 156-164.

Hofer, M.A., and Reiser, M.F. The development of cardiac rate regulation in preweanling rats. Psychosom. Med. 1969, 31, 372-388.

Iams, S.G., McMurtry, J.P., and Wexler, B.C. Aldosterone, deoxycorticosterone, corticosterone, and prolactin changes during the lifespan of chronically and spontaneously hypertensive rats. Endocrinology 1979, 104, 1357-1363.

Jern, S., Sivertsson, R., and Hansson, L. Possible relationship between psych-emotional factors and haemodynamic patterns in the pathogenesis of mild blood pressure elevation. Clin. Sci. 1981, 61, 93s-95s.

Jose, A.D. Effect of combined sympathetic and parasympathetic blockade on heart rate and cardiac function in man. Am. J. Cardiol. 1966, 18, 476-478.

Julius, S., and Schork, M.A. Borderline hypertension - a critical review. J. Chronic. Dis. 1971, 23, 723-754.

Kalimi, M. Comparison of glucocorticoid receptors in various tissues of adult and senescent rats. Gerontology 1982, 28, 371-376.

Kant, G.J., Bunnell, B.N., Mougey, E.H., Pennington, L.L., and Meyeroff, J.L. Effects of repeated stress on pituitary cyclic AMP, and plasma prolactin, corticosterone, and growth hormone in male rats. Pharmacol. Biochem. Beh. 1983, 18, 967-971.

Keller, S., and Seraganian, P. Physical fitness level and autonomic reactivity to psychosocial stress. J. Psychosom. Res. 1984, 28, 279-287.

Kopin, I.J., McCarty, R., and Yamaguchi, I. Plasma catecholamines in human and experimental hypertension. Clin. Exp. Hypertension 1980, 3-4, 379-394.

Kudo, Y., Sokabe, H., and Kawashima, K. Effects of acute and chronic treatments with atenolol and propranolol on cardiovascular responses to handling stress in spontaneously hypertensive rats. J. Pharm. Dyn. 1983, 6, 729-736.

Kvetnansky, R., McCarty, R., Thoa, N.B., Lake, C.R., and Kopin, I.J. Sympatho-adrenal responses of spontaneously hypertensive rats to immobilization stress. Am. J. Physiol. 1979, 236, H457-H462.

Kvetnansky, R., Sun, C.L., Lake, C.R., Thoa, N., Torda, T., and Kopin, I.J. Effect of handling and forced immobilization on rat plasma levels of epinepherine, norepinepherine, and dopamine-beta-hydroxylase. Endocrinology 1978, 103, 1868-1874.

Kvetnansky, R., and Mikulaj, L. Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. Endocrinology 1970, 87, 738-743.

Kvetnansky, R., Weise, V.K., and Kopin, I.J. Elevation of adrenal tyrosine hydoxylase and phenylethanolamine-N-methyl transferase by repeated immobilization of rats. Endocrinology 1970, 87, 744-749.

Langer, P.W., Obrist, P.A., and McCubbin, J.A. Hemodynamics and metabolic adjustments during exercise and shock avoidance in dogs. Am. J. Physiol. 1979, 236, H225-H230.

Lawler, J.E., Barker, G.F., Hubbard, J.W., Cox, R.H., and Randall, G.W. Blood pressure and plasma renin activity responses to chronic stress in the borderline hypertensive rat. Physiol. Beh. 1984, 32, 101-105.

Lawler, J.E., Barker, G.F., Hubbard, J.W., and Schaub, R.G. Effects of stress on blood pressure and cardiac pathology in rats with borderline hypertension. Hypertension 1981, 3, 496-505.

Lawler, J.E., Barker, G.E., Hubbard, J.W., and Allen, M.T. The effects of conflict on tonic levels of blood pressure in the genetically borderline hypertensive rat. Psychophysiology 1980, 17, 363-370.

Lenox, R.H., Kant, G.J., Sessions, G.R., Pennington, L.L., Mougey, E.H., and Meyeroff, J.L. Specific hormonal and neurochemical responses to different stressors. Neuroendocrinology 1980, 30, 300-308.

Lewis, S.F., Nylander, E., Gad, P., and Areskog, N. Non-autonomic component in bradycardia of endurance trained men at rest and during exercise. Acta Physiol. Scand. 1980, 109, 297-305.

Lin, Y., and Horvath, S.M. Autonomic nervous control of cardiac frequency in the exercise-trained rat. J. Appl. Physiol. 1972, 33, 796-799.

Lundgen, Y., Hallback, M., Weiss, L., and Folkow, B. Rate and extent of adaptive cardiovascular changes in rats during experimental renal hypertension. Acta Physiol. Scand. 1974, 91, 103-115.

McCarty, R., Chiueh, C.C., and Kopin, I.J. Behavioral and cardiovascular responses of spontaneously hypertensive and normotensive rats to inescapable footshock. Beh. Biol. 1978, 22, 405-410.

McCarty, R., and Kopin, I.J. Changes in plasma catecholamines and behavior of rats during the anticipation of footshock. Hormones Beh. 1978a, 11, 248-257.

McCarty, R., and Kopin, I.J. Sympatho-adrenal medullary activity and behavior during exposure to footshock stress: A comparison of seven rat strains. Physiol. Beh. 1978b, 21, 567-572.

McCarty, R., Kvetnansky, R., Lake, C.R., Thoa, N.B., and Kopin, I.J. Sympatho-adrenal activity of SHR and WKY rats during recovery from forced immobilization. Physiol. Beh. 1978, 21, 951-955.

Mackay-Sim, A. Discrimination of odors from stressed rats by non-stressed rats. Physiol. Beh. 1980, 24, 699-704.

Mackay-Sim, A., and Laing, D.G. Rats' responses to blood and body odors of stressed and non-stressed conspecifics. Physiol. Beh. 1981, 27, 503-510.

Malhotra, A., Penpargkul, S., Schiable, T., and Scheuer, J. Contractile proteins and sarcoplasmic reticulum in physiologic cardiac hypertrophy. Am. J. Physiol. 1981, 241, H263-H267.

Manuck, S.B., Giordani, B., McQuaid, K.J., and Garrity, S.J. Behaviorally-induced cardiovascular reactivity among sons of reported hypertensive and normotensive parents. J. Psychosom. Res. 1981, 25, 261-269.

Manuck, S.B., Proietti, J.M., Rader, S.J., and Polefrone, J.M. Parental hypertension, affect, and cardiovascular response to cognitive challenge. Psychosom. Med. 1985, 47, 189-200.

Mason, J.W., Maher, J.T., Hartley, L.H., Mougey, E.H., Perlow, M.J., and Jones, L.G. Selectivity of corticosteroid and catecholamine responses to various natural stimuli. In: G. Serban (Ed.). Psychopathology of human adaptation. New York: Plenum, 1976.

Matlina, E. Effects of physical activity and other types of stress on catecholamine metabolism in various animal species. J. Neural Trans. 1984, 60, 11-18.

Meehan, JP. Stress, vascular changes and the potential for behavioral modification. J. S. C. Med. Assoc. 1983, 79, 535-538.

Messerli, F.H., Frohlich, E.D., Suarez, D.H., Reisen, E., Dreslinski, G.R., Dunn, F.G., and Cole, F.E. Borderline hypertension: relationship between age, hemodynamics, and circulating catecholamines. Circulation 1981, 64, 760-764.

Millenson, J.R., and Dent, J.G. Habituation of conditioned suppression. Quart. J. Exp. Psych. 1971, 23, 126-134.

Miller, N.E. A perspective on the effects of stress and coping on disease and health. In: S. Levine & H. Ursin (Eds.). Coping and health. New York: Plenum, 1980. Pp. 323-354.

Milnor, W.R. Autonomic and peripheral control mechanisms. In: V.B. Mountcastle (Ed.). Medical Physiology, 14th ed. New York: C.V. Mosby, 1980a. Pp. 1047-1060.

Milnor, W.R. The cardiovascular control system. In: V.B. Mountcastle (Ed.). Medical physiology, 14th ed. New York: C.V. Mosby, 1980b. Pp. 1089-1093.

Natelson, B.H., Tapp, W.N., Adamus, J.E., Mittler, J.C., and Levin, B.E. Humoral indices of stress in rats. Physiol. Beh. 1981, 26, 1049-1054.

Natelson, B.H., Krasnagor, N., and Holaday, J.W. Relations between behavioral arousal and plasma cortisol in monkeys performing repeated free-operant avoidance sessions. J. Comp. Physiol. Psych. 1976, 90, 958-969.

Obrist, P.A. Cardiovascular psychophysiology. New York: Plenum, 1981. Pp. 198-200.

Okamato, K. (Ed.). Spontaneous hypertension: its pathogenesis and complications. Tokyo: Igaiku Shoin, 1972.

Paul, O., Lepper, M.H., Ostfeld, A., MacMillan, A., and Phelan, W.H. Coronary heart disease in an industrial population: A prospective study. Circulation 1962, 26, 770-789.

Pavlik, G., and Frenkl, R. Sensitivity to catecholamines and histamine in the trained and in the untrained human organism and sensitivity changes during digestion. Eur. J. Appl. Physiol. 1975, 34, 199-204.

Peronnet, F., Nadeau, R.A., deChamplain, J., Magrassi, P., and Chatrand, C. Exercise plasma catecholamines in dogs: Role of adrenals and cardiac nerve endings. Am. J. Physiol. 1981, 241, H243-H247.

Raab, W., and Krzywanek, H.J. Cardiovascular sympathetic tone and stress response to personality patterns and exercise habits. Amer. J. Cardiol. 1965, 16, 42-53.

Ring, G., Dupuch, G., Creed, J. Effect of voluntary exercise on the resting oxygen consumption in rats. Gerontologia 1967, 13, 194-199.

Robinson, S. Physiology of muscular exercise. In: V.B. Mountcastle (Ed.). Medical physiology, 14th Ed. New York: C.V. Mosby, 1980. Pp. 1387-1416.

Robinson, B.F., Epstein, S.E., Beiser, G.D., and Braunwald, E. Control of heart rate by the autonomic nervous system. Circ. Res. 1966, 19, 400-411.

Rosenman, R.H., Friedman, M., Straus, R., Wurm, M., Kositch, J., Hahn, W., and Werthessen, N.T. A predictive study of coronary heart disease. J. A. M. A. 1964, 189, 15-26.

Scheuer, J., and Tipton, C.M. Cardiovascular adaptations to physical training. Ann. Rev. Physiol. 1977, 39, 221-251.

Schaible, T.F., and Scheuer, J. Cardiac function in hypertrophied hearts from chronically exercised female rats. J. Appl. Physiol. 1981, 50, 1140-1145.

Schulte, W., and Neus, H. Hemodynamics during emotional stress in borderline and mild hypertension. Eur. Heart J. 1983, 4, 803-809.

Seggie, J.A., and Brown, G.M. Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat, following handling or exposure to novel environment. Can. J. Physiol. Pharmacol. 1975, 53, 629-637.

Selye, H. A syndrome produced by several nocuous agents. Nature 1936, 138, 32-37.

Selye, H. The stress of life (2nd Ed.). New York: McGraw Hill, 1976.

Smith, L.C., and Dugal, L.P. Age and spontaneous running activity of male rats. Can. J. Physiol. Pharmacol. 1965, 43, 852-856.

Smith, T.L., and Hutchins, P.M. Anesthetic effects on hemodynamics of spontaneously hypertensive and Wistar-Kyoto rats. Am. J. Physiol. 1980, 238, H539-H544.

Smith, T.L., and Hutchins, P.M. Central hemodynamics in the developmental stage of spontaneous hypertension in the anaesthetized rat. Hypertension 1979, 1, 508-517.

Smith, P.G., Poston, C.W., and Mills, E. Ontogeny of neural and non-neural contributions to arterial blood pressure in spontaneously hypertensive rats. Hypertension 1984, 6, 54-60.

Smookler, H.H., and Buckley, J.P. Relationships between brain catecholamine synthesis, pituitary adrenal function, and the production of hypertension during prolonged exposure to environmental stress. Int. J. Neuropharm. 1969, 8, 33-41.

Starzec, J.J., Berger, D.F., and Hesse, M.S. Effects of stress and exercise on plasma corticosterone, plasma cholesterol, and aortic cholesterol levels in rats. Psychosom. Med. 1983, 45, 219-226.

Steinhaus, A.H. Chronic effects of exercise. Physiol. Rev. 1933, 12, 103-147.

Suzuki, S., Oshima, S., and Higuchi, M. Influence of physical exercise and nutrition on blood pressure of SHR. Jpn. Heart J. 1979, 20(Suppl. I), 365-370.

Tipton, C.M. Exercise, training, and hypertension. Exerc. Sport. Sci. Rev. 1984, 12, 245-306.

Tobian, L. Jr., Janecek, J., Tomboulian, A., and Ferreira, D. Sodium and potassium in the walls of arterioles in experimental renal hypertension. J. Clin. Invest. 1961, 40, 1922-1925.

Weiner, H. Psychobiology of essential hypertension. New York: Elsevier, 1979. Pp. 41-121.

Weiss, J.M., Bailey, W.H., Pohorecky, L.A., Korzeniowski, D., and Grillione, G. Stress-induced depression of motor activity correlates with regional changes in brain norepinepherine but not in dopamine. Neurochem. Res. 1980, 5, 9-21.

Weiss, L. Aspects of the relation between functional and structural factors in primary hypertension. Acta Physiol. Scand. 1974, 409, 1-58.

Winters, W.G., Leaman, D.M., and Anderson, R.A. The effect of exercise on intrinsic myocardial performance. Circulation 1973, 48, 50-55.