To the Graduate Council:

I am submitting herewith a dissertation written by Shenggang Li entitled "Renal Sympathetic Nerve Activity and Cardiovascular Responses to Classical Conditioning and High Dietary Sodium Intake in Rats." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Life Sciences.

Dr. James E. Lawler, Major Professor

We have read this dissertation and recommend its acceptance:

Kathleen A. Lawler

Accepted for the Council:

Associate Vice Chancellor and
Dean of The Graduate School

RENAL SYMPATHETIC NERVE ACTIVITY AND CARDIOVASCULAR RESPONSES TO CLASSICAL CONDITIONING AND HIGH DIETARY SODIUM INTAKE IN RATS

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Shenggang Li

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DEDICATION

To my grandfather LI Dao-Qian an ordinary man with great personality and to my grandmother HU Xou-Zhen who taught me to be tolerant to others.

To my father LI Qi-Bin and my mother XIONG Gu-Xiang who believe that nothing is worth more than education and tried their best to support me.

To my wife LIU Li for her love, encouragement and sacrifice and to my son Winston who always reminds me life is good and tomorrow will be better.

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ABSTRACT

Evidence from electrical stimulation and denervation studies suggests that the renal nerves contribute greatly to hypertension in many experimental models of this disease and appear to play a role in human hypertension as well. However, the interaction between renal sympathetic nerve activity (RSNA) and cardiovascular function during behavioral stress and the effect of high salt diet on these measures has been poorly documented. Two experiments were conducted to investigate differences in RSNA, mean arterial pressure (MAP) and heart rate (HR) responses to classical conditioning in rats differing in genetic predisposition to hypertension and in rats subjected to high dietary salt intake.

A total of 48 animals, 12 spontaneously hypertensive rats (SHR), 12 normotensive Wistar-Kyoto (WKY) rats and 24 borderline hypertensive rats (BHR) at 12-14 weeks of age, were studied in the conscious state during discriminative conditioning. One half (N=12) of the BHR were subjected to

8% high salt diet for 8 weeks prior to measurement. Animals were given 5 days of habituation and conditioning with 5 trials of pulsed tone (eventually to become the positive conditioning stimulus, or the CS+) that was associated with tail-shock and 5 trials of an otherwise identical, but continuously-sounded tone (eventually to become the negative conditioning stimulus, or CS-) that was never associated with tail-shock, both 15 sec in duration. After a 48 hour recovery period from the catheterization and renal nerve electrode implantation, RSNA, MAP and HR were measured continuously for ten conditioning trials (5 CS+ and 5 CS- trials, each 30 sec in length). Cardiovascular and renal measurements for each trial included a 9 sec baseline, a 15 sec tone presentation period, and a 6 sec period following the termination of the tone.

A similar pattern of responses in RSNA, HR and MAP was observed in all group of animals during each trial type, but the magnitude of the responses differed depending on group and trial type. Overall, tone onset caused an abrupt sudden burst of RSNA which was followed by an increase in HR and MAP within .5 sec. This was followed by a quiet neural

traffic period and a bradycardic/depressor response. This pattern suggests an increase in parasympathetic and a decrease in sympathetic activity through a baroreflex control mechanism. A sustained pressor/bradycardic response consistent with higher sympathetic and parasympathetic activity were also observed in all animals in the remaining seconds of the tone period.

With regard to the influences of genetic predisposition, it was concluded that (1) genetic predisposition determines baseline MAP, but not HR or RSNA levels; (2) genetic predisposition determines reactivity of RSNA, HR and MAP to stress; (3) the RSNA, MAP and HR responses to the two trial types (CS+ and CS-) can be effectively discriminated and animals can establish behavioral responses to stress through a learning process; (4) SHR may have a higher receptor sensitivity to norepinephrine and epinephrine, and/or a vascular defect when compared to WKY and BHR; (5) though SHR, BHR and WKY have comparable total baroreflex gain in MAP in response to conditioned and unconditioned stimuli, the set point is shifted differently, depending on their genetic

predisposition, to a higher level during continued conditioning stimulation; and (6) sympathetic activity is dominant over parasympathetic activity in blood pressure regulation during continued conditioning stimulation.

The effect of high dietary sodium (8% NaCl) intake was tested only in the BHR strain. From these results, it was concluded that (1) high salt diet increases baseline MAP, but not HR or RSNA; (2) high salt diet does not change the general pattern of responses in RSNA, HR, and MAP to classical conditioning; (3) high salt diet enhances the learned responses in RSNA to behavioral stress during sudden burst and tone period; (4) high salt diet enhances the conditioned response in MAP; however, the enhanced pressor response appears to be diminished by the baroreflex during the tone period; (5) the unconditioned responses and startle responses in MAP and HR are not affected differentially by dietary sodium intake; and (6) it seems that high salt diet does not change the tonic level or reactivity of heart rate in BHR.

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LIST OF ABBREVIATIONS

$RSNA_{rest}$	Baseline Renal Sympathetic Nerve Activity
$RSNA_{sb}$	Renal Sympathetic Nerve Activity during Sudden Burst Period
$\Delta RSNA_{sb}$	Percent Change of Sudden Burst Renal Sympathetic Nerve Activity from Baseline
$RSNA_{qn}$	Renal Sympathetic Nerve Activity during Quiet Neural Traffic Period
$\Delta RSNA_{qn}$	Percent Change of Renal Sympathetic Nerve Activity from Baseline during Quiet Neural Traffic Period
$RSNA_{tone}$	Renal Sympathetic Nerve Activity during the Tone Period (seconds 15.00-23.99)
$\Delta RSNA_{tone}$	Percent Change of Renal Sympathetic Nerve Activity from Baseline during the Tone Period
SBP	Systolic Blood Pressure
DBP	Diastolic Blood Pressure
MAP	Mean Arterial Pressure
MAP_{rest}	Baseline Mean Arterial Blood Pressure
MAP_{c1}	Mean Arterial Pressure Change from Baseline during First Conditioned Response
MAP_{qp}	Mean Arterial Pressure change from Baseline during the Depressor Period following First Conditioned Response
MAP_{tone}	Mean Arterial Pressure Changes from Baseline during the Tone Period (seconds 15.00-23.99)
HR_{rest}	Baseline Heart Rate

HR_{c1}	Heart Rate Change from Baseline during First Conditioned Response Period
HR_{qp}	Heart Rate Change from Baseline during the Bradycardic Response
HR_{tone}	Heart Rate Change from Baseline during the Tone Period (seconds 15.00-23.99)
CS+	Positive Conditioning Stimulus (Pulsed Tone)
CS-	Negative Conditioning Stimulus (Non-Pulsed Tone)
UC	Unconditioned Stimulus (Tail-Shock)
UCR	Unconditioned Response

CHAPTER I

INTRODUCTION

There are 50 million adults in the United States with hypertension, defined as a systolic blood pressure (SBP) of 140 mmHg or higher, a diastolic blood pressure (DBP) of 90 mmHg or higher, or a level requiring antihypertensive medication. This was reported by in the National Health and Nutrition Examination Surveys after the third national survey of a large representative sample of the U.S. adult population during 1988-1991 (NHANES, 1993). This is nearly 20% of the entire U.S. population at any point in time. Moreover, when considering the late onset of the disease, most people will develop hypertension at some point during their lifetime. Although it is often benign in itself, hypertension is implicated in many clinical problems such as atherogenesis of large arteries and in non-sclerotic lesions and fibrinoid necroses of the small arteries and arterioles. As a result, hypertension-influenced diseases remain the most common causes of mortality in developed societies.

Some hypertensive cases are secondary to conditions such as pyelonephritis, pheochromocytoma and coarctation of the aorta, but the majority of cases (more than 90%) are not associated with specific diseases, and are classified as essential or primary (Danielson and Dammstrom, 1981; Sinclair et al, 1987). However, authorities still debate three basic problems of essential hypertension: the etiology of the disease, its pathological mechanisms, and the level of blood pressure considered abnormal.

The first debate is about the etiology of hypertension. Fifty years ago, almost all hypertension not obviously secondary to intrinsic renal disease was classified as "essential." Since then, a small percentage of hypertensive diseases, such as Cushing's syndrome, renovascular disease, and primary aldosteronism etc., have been identified. It is still possible that an additional part of what is now considered essential will be separated into other specific categories. There are considerable variations in the role of the various genetic and environmental factors at different times and stages of hypertension and in different people. For example, hypertensives may be subclassified with respect

to their renin activity and sodium sensitivity, etc. However, no basis for division into different diseases is apparent at present.

Several genetic and environmental factors cause individuals to develop sustained hypertension, but they do not apply in all cases. The evidence for genetic involvement is considerable. Estimates of the genetic contribution to the variability of blood pressure vary from 25% in pedigree studies to 65% in twin studies (Williams et al, 1991). Whatever the proportion, it is clear that environmental factors (such as dietary sodium intake or psychological stress) must make a substantial contribution. We will discuss these factors in detail in subsequent sections.

The second debate has concerned pathological mechanisms. Two major theories have been proposed. It is recognized that arterial pressure is the dynamic consequence of many short-term and long-term factors that can influence cardiovascular function. Therefore, it seems logical that sustained high blood pressure results from the dysfunction of the long-term regulation systems. At present, two general

concepts prevail regarding long-term pressure regulation, the "neural theory" and the "fluid theory". The "neural theory" suggests that the nervous system detects changes in arterial pressure and provides both rapid stabilization and long-term control of pressure predominantly by adjusting levels of sympathetic tone (Abboud, 1982; Chalmers, 1975; Folkow, 1993; Julius, 1988; Obrist, 1979). The "fluid theory" suggests that the long-term regulation of pressure is achieved by the slow adjustment of body fluid volumes based on the ability of the kidneys to respond directly to changes of arterial pressure by the mechanism of pressure diuresis (Cowley, 1992; Guyton, 1992; Wardner, 1990a and 1990b).

Based on this differentiation, psychological stress would be expected to influence cardiovascular function predominantly or directly through the sympathetic nervous system, whereas dietary sodium would be more directly involved in the regulation of fluid homeostasis. However, the nervous system also influences and interacts with the fluid volume regulators. So the differences between two concepts can become indistinct. Studies have also

demonstrated that sympathetic activation in patients with early essential hypertension involves the renal vascular beds to a greater degree than other vascular beds (e.g., Brown et al, 1981). As the key organs of fluid volume control, the kidneys themselves are under precise central sympathetic control (Barajas et al, 1992; DiBona, 1985). The renal nerves contribute to hypertension in many experimental models, and appear to play a role in human hypertension as well (Lawler et al, 1989; Leeuw et al, 1987; Thoren and Ricksten, 1979; Winternitz et al, 1980). The role of the renal nerves in hypertension will be described subsequent sections.

The third debate has been the level of blood pressure considered abnormal, in other words, the dividing line between normal and high. Pickering for many years challenged the wisdom of that debate and decried the search for that arbitrary dividing line (Pickering, 1967). He suggested that "the division of arterial pressure into normotension and hypertension is, scientifically speaking, in the nature of an artifact" (p. 18). Blood pressure is a dynamic rather than fixed parameter, and levels vary considerably over the

day. This has been noted even with the same well-trained observer using a calibrated sphygmomanometer under controlled circumstances (Watson et al, 1987). Furthermore, the classification of a reading as high depends on the age of the individual, since arterial pressures tend to rise as people grow older (Rowland and Roberts, 1982).

Results from a meta-analysis of all available major prospective observational studies relating DBP level to the incidence of coronary heart disease estimate that a 5.0 mmHg increase in DBP is associated with at least a 34% increase in stroke risk and at least a 21% increase in coronary heart disease risk. However, there is no evidence of any threshold level of DBP below which lower levels of DBP were not associated with lower risks of stroke and coronary heart disease (MacMahon et al, 1990). The definition of hypertension as SBP/DBP equal to or higher than 140/90 mmHg, as recommended by the Fifth Joint National Committee on Detection, Evaluation and Treatment of High Blood Pressure (Joint National Committee, 1993), is obviously arbitrary. However, it serves the purpose for medical practice that some criteria be used to determine the need for workup and

therapy. Since the dividing criteria are mostly issues of clinical or epidemiological relevance, we will not discuss them further here.

I. GENETIC PREDISPOSITION TO HYPERTENSION

Two classes of variables determine the phenotype of every individual: genetic factors and environmental influences. For a particular property, either factor may exert the sole or predominant effect: both classes of factors may act independently, or there may be an interaction between certain genes and certain environmental factors, the nature of which can range from relatively simple to exceedingly complex. The latter is considered by the majority of investigators to be the case in essential hypertension (Dominiczak and Lindpaintner, 1994; Morris, 1993). In contrast to single gene disorders, levels of blood pressure do not generally segregate in families as monogenic traits, i.e., the results of most studies indicate that variation in blood pressure is due to the combined effects of multiple genes and environment (Dominiczak and Lindpaintner, 1994). As a result, different individuals,

even within the same family, may have hypertension caused by different combinations of these genetic and environmental factors. While the epidemiological observations can estimate the role of genetic factors in hypertension at large, the use of the new tools of molecular biology promises to uncover the basic genes and mechanisms involved. Different hypertensive animal models represent a variety of genetic predispositions to the disease.

Epidemiological Studies

The most convincing epidemiological evidence showing the hereditary influence on essential hypertension comes from twin studies. Platt (1967) found that monozygotic twins developed hypertension at the same age as their twins even though they had lived apart for many years. The intraclass correlation coefficient is used as an index to show the influences of genetic vs. environmental factors over a certain phenotype. Feinleib et al (1977) found that the intraclass coefficients for DBP were .58 for 250 pairs of monozygotic twins and .27 for 264 pairs of dizygotic twins, all adult white males. Similar results were reported on 197 pairs of twin seven-year-old schoolchildren with intraclass

coefficients of .54 for monozygotic and .27 for dizygotic pairs (Havlik et al, 1979).

One must recognize that there is no perfect way of clearly separating the effects of genetic factors from environmental influences based on epidemiological data, since twins and siblings share not only similar genetic predispositions but also the same developmental environment. However, two concepts, "heritability" and "liability," help to estimate the degree of genetic influences on hypertension as well as other genetic characteristics. Falconer (1981) proposed to use "liability" as a genetic concept to express the whole combination of external (environmental) and genetic circumstances that makes an individual more or less likely to develop a disease. Heritability is described as a proportion of the additive genetic variance out of the phenotypic variance. The heritability (H^2) can be estimated by the equation, $H^2=b/r$ where b is the regression coefficient of relatives on propositi (i.e., the person in a family tree who was first found having the genetic disease) and r is the coefficient of relationship. For monozygotic twins r=1.0 and for the first-degree relatives r=.5. Using

this method, Knudson et al (1973) calculated that heritability was .76 for systolic pressure based on measurements on 23 monozygotic twins from three studies. The regression coefficients of blood pressure calculated by Mial and Oldham (1963) for first-degree relatives was .3, which would correspond to a heritability factor of .6 in that population.

Finleib et al (1977) used two heritability estimate methods on a data set of 1046 subjects (including 1028 twins). One method was based on variance analysis as shown above and another on the traditional estimate formula $H^2=2(P_{mz}-P_{dz})$, where P_{mz} equals intraclass correlation coefficient for monozygotic twin and P_{dz} equals intraclass correlation coefficient for dizygotic twin. The estimated heritability for DBP were .64 and .61 based on the two methods described above, respectively.

Essential hypertension clusters in families. For example, individuals with two or more first-degree relatives with hypertension before age 55 have a 3.8 times greater risk of developing hypertension before age 50 (Williams et

al, 1991). After investigating 350 siblings of 178 patients with severe hypertension, Platt (1967) estimated that the sibling had eight times the risk of developing a DBP of 110 mmHg or higher in middle age than a person selected at random.

Evolution is a relatively slow process, so gene pools vary among populations while they are relatively stable within a population. Therefore, one would expect that the incidence of the disease would vary in different population groups. This has been observed in many studies (e.g., Epstein, 1967; Liu et al, 1989). Epstein (1967) reviewed 34 different studies in various areas of the world by geographic area and plotted the populations into a figure with three SBP intervals of 105-115 mmHg, 116-125 mmHg, and 126-135 mmHg and five different grids of slopes (0-4), representing increasing rates of rise with age. That figure shows that there are differences in SBP between racial groups, i.e., the highest level and the steepest slope curve was found in Georgia-blacks, with more gradual levels and slopes among Georgia-whites. Finally, the lowest levels and slopes were observed in Caracas Indians and some

agricultural countries or areas. In a recent baseline examination of a longitudinal study involving 5116 young black and white men and women aged 18-30, blacks had higher mean systolic and diastolic pressures than whites did (Liu et al 1989). As this population was followed for 5 years, the black-white differences increased. Even after adjustments for age, education, body mass index, physical activity, and alcohol intake, 60% of the differences still exist (Liu et al, 1992). Although the role of some environmental factors (e.g., salt intake) was not excluded, these results suggest that there are differences in genetic predisposition to hypertension among ethnic groups.

Experimental Genetics of Hypertension

There has been a long debate about what type of inheritance is involved in essential hypertension (Platt, 1967; Pickering, 1967). For example, hypertension caused by the syndrome of glucocorticoid-remediable aldosteronism was once classified as "essential." However, this disease has been found to have a single mutation with Mendelian autosomal dominant transmission (Lifton et al, 1992). Based on the fact that blood pressure is a quantitative and

variable phenotype, involving many regulatory systems, and is influenced by environmental factors, essential hypertension is generally considered as a polygenetic, rather than single gene, disease (Morris, 1993).

There are two principal strategies or levels of approach towards the genetic etiology of hypertension, the observational, or case control study, and the molecular genetic approach. Case control studies search for the intermediate phenotypic traits (markers) while the molecular genetic approach tries to identify the genes involved.

Case Control Study. As a result of polygenetic and multifactor involvement, different individuals, even within the same family, may be hypertensive due to different combinations of these genetic factors and environmental triggers. This suggests that blood pressure measurement itself is not a sufficient physiological phenotype (marker) for the searching of hypertensive genotype (genes). The case control study strategy looks for any intermediate phenotypic traits or markers correlating with blood pressure. In other words, by comparing a hypertensive group and a control

group, it seeks any physiological or biochemical changes associated with blood pressure changes either randomly or causally. These intermediate phenotypic traits can be renal nerve activity, levels of some enzymes, hormones, or neurotransmitters, etc. In the past, using epidemiological, physiological, biochemical, and other tools, case control studies have yielded a tremendous amount of informative data related to the etiology of hypertension. For example, four distinguishing characteristics were found in the normotensive offspring whose parents had higher pressures: higher levels of plasma angiotensinogen, cortisol, and 18-OH-corticosteroid, and homozygosity for the glucocorticosteroid receptor (Watt et al, 1992).

Further, the intermediate phenotypic differences between hypertensive and control groups or strains should be subjected to cosegregation analysis. A cosegregation, appearing or disappearing simultaneously or proportionally, between an intermediate phenotypic trait (e.g., angiotensin converting enzyme) and blood pressure supports, but does not prove, a causal relationship rather than a random association between them. Among the intermediate phenotypic

traits that have been shown to cosegregate with blood pressure are vascular smooth muscle responses to cations and oscillatory activity of mesenteric resistance arteries in spontaneously hypertensive rat (SHR), norepinephrine-induced oscillatory activity, and increased lymphocyte potassium efflux in the stroke-prone spontaneously hypertensive rat, increased adrenal production of 18-hydroxy deoxycorticosterone in Dahl rats, and increased red blood cell Na^+-K^+ co-transport in the Milan hypertensive strain (Dominiczak et al, 1994).

Although the case control study strategy has yielded valuable insights about blood pressure regulation and hypertension and despite the continuously growing need for employing this strategy, it yields only observational data. The etiological inference of this strategy is speculative since it can not distinguish between primary (causal) and secondary (resultant) events. Until recently, investigators taking the approach of correlating these intermediate phenotypes with blood pressure found it difficult to take matters further. Practically, it is impossible to demonstrate that a cosegregating intermediate phenotypic

trait is inherited and thus causally related to hypertension. A molecular genetic approach can address these problems.

Molecular Genetic Approaches. The use of new tools such as recombinant DNA technology and DNA sequence analysis promises to unravel the complexity of the genetics of hypertension in ways not possible with observational techniques or case control studies. Genetic differences that may be responsible for hypertension are defined in terms of differences in DNA structure, or polymorphism, between hypertensive and normotensive animals. Two molecular genetic approaches are commonly taken to identify these differences, candidate gene cosegregation analysis and random marker genetic mapping.

Candidate Gene Cosegregation Approach. In this approach, a gene encoding a protein (e.g., angiotensin converting enzyme) with a known or suspected physiological role in the regulation of cardiovascular function is examined for the transgenerational maintenance of association of a given variant (polymorphism) with

hypertension. One basic method of the candidate gene approach is restriction fragment length polymorphism analysis. It utilizes restriction enzymes to digest the genomic DNA and into specific sequences in a gene of interest. If a polymorphism exists between the two strains, the restriction enzyme will cut at different sites and the resulting DNA fragments will differ in size. In other words, first one identifies a gene of a suspected intermediate phenotypic trait with DNA sequence analysis, then identifies a polymorphic marker on that gene, and finally seeks cosegregation between blood pressure levels and that polymorphic marker.

The genes for renin, angiotensin (I and II) and angiotensin converting enzyme have all been mapped and cloned (Corvol et al, 1989). Also, the presence of increased plasma angiotensinogen fits nicely with the evidence for genetic cosegregation between a variant angiotensinogen gene and hypertension found in 379 hypertensive sibling pairs in Jeunemaitre's study (Jeunemaitre et al, 1992a). More notably, three independent rat F2 populations have been tested for cosegregation between blood pressure and a

polymorphic marker on the renin gene. In one study, performed on a cross between the Dahl salt-sensitive and salt-resistant strains, the renin gene locus has been found to be linked to hypertension (Rapp et al, 1989), whereas in a cross between stroke-prone spontaneously hypertensive rat and WKY, no such effect was present (Lindpaintner et al, 1990). In another study, performed on a cross between SHR and Lewis rats, an association was found between the presence of the hypertensive renin allele and blood pressure in animals heterozygous, but not homozygous, at the renin locus (Kurtz et al, 1990).

The major limitation of candidate gene analysis lies in the fact that candidate genes are, by necessity, derived from the relatively small pool of genes recognized so far (3,000-4,000), compared with the estimated total number of genes in the mammalian genome (100,000). The disease-relevant genes may not be found by this approach for many years, since they can be among the 97% unknown rather than among the 3% known genes (Dominiczak et al, 1994).

Random Marker Genetic Mapping. This approach is based

on the use of functionally unrelated polymorphic genetic markers to localize genes of interest by virtue of their spatial association. Due to meiotic recombination events, only markers that are physically in close proximity to the responsible gene will demonstrate a high degree of association, or linkage. In other words, only those alleles that contribute to the trait are expected to show cosegregation; this distinguishes them from the large number of other polymorphic markers that are characteristic for the genetic makeup of each strain but do not reside in the proximity of disease-related genes. An experiment conducted in a cross between stroke-prone spontaneously hypertensive rat and WKY is noteworthy for the finding of linkage between a region on rat chromosome 10 and SBP and DBP after dietary sodium loading (Hilbert et al, 1991). The marker originally used for the identification of this linkage was found to be closely associated with the gene for angiotensin converting enzyme by syntony analysis with human linkage groups.

This random mapping approach screens the entire genome of animals rather than a few suspected candidate genes. So it is powerful in searching for unknown disease genes.

However, this approach identifies only a chromosomal region. The identification of a genetic marker linked with morbid phenotype represents only the first and the simplest step in the identification of a hypertensive gene(s). The success of genetic mapping studies depends on many factors, among them the genetic makeup of the cross, the fidelity of measurements and parameters chosen for phenotyping, and technical aspects regarding the analytical work.

The identification of specific genetic linkages and mutations will only point the way to aberrant physiologic pathways, so that the integration of observational data will still be needed. For example, the findings of cosegregation or genetic linkage between the angiotensinogen gene and hypertension (Jeunenmaitre et al, 1992) and the presence of increased levels of plasma angiotensinogen in subjects with higher blood pressure or subjects with parental hypertensive history (Watt et al, 1992) only support a role for increased synthesis of angiotensinogen in hypertension. These data do not explain how this translates into elevated blood pressure. Nor can they prove that the angiotensin system is either necessary or sufficient in hypertension development.

A causative role of angiotensin converting enzyme has never been demonstrated despite many years of research on protein level.

The relevance of data from animal models is highly questionable in terms of hypertension-causing genes. Every single one of the genetic differences present between disease and control strains (i.e., SHR and WKY rats) represents a polymorphic marker that distinguishes between strains. However, only a tiny fraction, representing disease-related genes, are of interest to the investigator. Taking hypertension in the SHR as an example, only an estimated three to six loci (Rapp, 1983) of a purported $5x10^7$ potential polymorphic sites (Johnson et al, 1992) will represent blood pressure-relevant markers. This implies that differences observed on any level (physiological, biochemical, or molecular genetic) between the hypertensive and the normotensive strains are much more likely to be unrelated than related to hypertension.

Furthermore, the relevance of animal data to the human condition is suspect, though 70% of current research on

hypertension is being done on rats or other animals (Carretero et al, 1991). For example, linkage of blood pressure to the angiotensin-converting enzyme locus has been noted in the SHR (Jacob et al, 1991; Hilbert et al, 1991), but no such linkage has been found in human hypertension (Jeunemaitre et al, 1992b; Harrap et al, 1993; Schmidt et al, 1993).

Although a lot of progress has been made toward understanding the molecular genetics of hypertension, caution must be taken when interpreting these data. The continued need for integrated study is even more obvious given the multifactorial nature of hypertension. "Putting all the bits together is crucial for real understanding of 'who are we'" (Folkow, 1994; Pg. 93).

Animal Models of Hypertension

It is of great interest to find out exactly which genetically linked deviations contribute to essential hypertension and also how they interact, first in animal models and then in humans, with other factors. Advantages of animal models include the compressed life span, ease of

access for experimental analysis, and control over genetic and environmental factors. We expect to unmask the inherited predisposing elements in animal models by controlling environmental influences, such as diet, and physical and psychological stresses. As mentioned previously, 70% of current research on hypertension is being done on rats or other small animals (Carretero et al, 1991).

Smirk and Hall (1958) were the first to breed a New Zealand spontaneously hypertensive rat with a small heart. This was soon followed by the Okamoto-Aoki spontaneously hypertensive rat (SHR) developed from its counterpart normotensive Wistar-Kyoto (WKY) rat (Okamoto et al, 1963). Dahl et al (1962) bred a salt-sensitive rat strain that became hypertensive only if fed excess salt. In 1980, Lawler and coworkers bred a borderline hypertensive rat (BHR) strain (Lawler et al, 1980).

Among these animal models, SHR and its counterpart WKY are being used more often than others. Young SHR rats showed greater increases in sympathetic nervous system activity (Koepke et al, 1985), plasma catecholamines (Ely et al,

1983), renal function (Koepke et al, 1985), and hemodynamics (McMurtry et al, 1981) than their WKY controls when subjected to high sodium diet, foot-shock, loud noises, vibrations, air jet stress, physical restraint, ether, heat, cold stress, etc.

One limitation of the SHR as an animal model of hypertension is that, unlike humans, it invariably develops severe blood pressure elevations unless it is socially isolated (Hallback, 1975). From this point of view, the BHR is an interesting, and perhaps more appropriate, animal model for human hypertension (Lawler et al, 1980; Sanders et al, 1992). The BHR is bred by mating the female SHR with the male WKY, so it represents a moderate genetic predisposition for essential hypertension (Lawler, 1980; Sanders, 1992). These F1 offspring have an average tailcuff SBP of 143 mmHg (s.e.=3.8), compared with 118 mmHg (s.e.=2.0) for WKY rat, and 171 mmHg (s.e.=3.8) for SHR, all on normal diet, not stressed, and directly measured at the age of 13 weeks (Lawler et al, 1987). Therefore, the advantages of this strain as a model of essential hypertension are that it naturally has a borderline high blood pressure and that it

is reported to be sensitive to both high salt diet and environmental stress (Sanders et al, 1992).

II. PSYCHOLOGICAL STRESS AND SYMPATHETIC NERVOUS SYSTEM ACTIVITY IN HYPERTENSION

Psychological stress influences cardiovascular function as well as many other behaviors such as sleep, arousal, mental activity, physical work, etc. Physiological responses to threatening environmental events were first studied by Cannon (1929), who observed that many physical and psychological stimuli caused the release of a hormonal agent from the adrenal glands into the blood, leading to a rise in blood pressure. Cannon called the humoral agent sympathin, which eventually was identified as a mixture of epinephrine and norepinephrine (Von Euler US, 1954). Several aspects of the role of psychological stressors in hypertension are being investigated, including (1) using acute or chronic stressors in either conditioning or non-conditioning paradigms to induce pressor responses or hypertension in animal models; (2) searching the physiological mechanisms involved in this process; and (3) identifying the

determinants, such as personality pattern, of cardiovascular reactivity to stress.

Stress in Non-conditioning Paradigms

There are more than a dozen physical or chemical stimuli which have been utilized, individually or in combination, as psychological stressors to induce hypertension. When an additional "neutral" stimulus is applied contingently (in the case of classical conditioning) or an avoidance response is allowed (in the case of operant conditioning), it is called a conditioning paradigm (Gormezano et al, 1967). Otherwise, it is called a non-conditioning paradigm. However, according to the new Pavlovian theory, any message (e.g., timing, background sound, light) happening before, during or after the presentation of an aversive stimulus may be a conditional stimulus though the investigators do not intentionally enforce or control it (Rescorla, 1988). Likewise, in a compound stress study, one stressor may be considered as a conditional stimulus to another, except that both stimuli are too aversive to be judged as "neutral" (CS).

Physical and Chemical Stimuli. Loud noise as a physical stimulus was utilized to induce hypertension half a century ago. Medoff et al (1945) exposed 31 rats to a constant air blast loud noise for 10 minutes daily, for 10 months. The frequency of the noise was not measured. Although not all animals had large increases in blood pressure, more than half (17) of the total animals tested showed at least one SBP reading greater than 180 mmHg.

Later, loud noises were used in combination with other stressors to induce hypertension. Hudak and Buckley (1961) tested the pressor response to combined stressors of 100 dB noise, flashing lights (150 w indoor reflector spot lights), and cage oscillation (60 times per minute) on 12 female Wistar rats (9 in stress, 3 in control group). Two stressors were randomly combined at any time and administrated 4 hours a day, 4 days a week, for a period of 35 weeks. The SBP of stressed animals gradually rose to a maximum of 151 mmHg while the SBP of control animals was maintained at around 110 mmHg. Some animals died before the study was completed. However, no cause of death was described.

The implications of Hudak and Buckley's study might be speculative due to the small number of animals tested and the lack of appropriate control groups. That procedure was revised in a study by Smookler and Buckley (1969). Rats were subjected to 20 weeks of randomized loud noises, flashing lights and motion stress. After 12 weeks of stress, SBP values reached approximately 160 mmHg. Since measurements were not taken during a post-stress period or followed after stress terminated, it is not known whether the hypertension produced by this manipulation was sustained. Perhach and coworkers (1975) tested such a possibility by using stressors similar to those employed by Smookler and Buckley (1969). SBP was sustained at 160 mmHg for 20 weeks following the termination of the stress.

Perhaps electrical shock is the most commonly used physical stimulus in both non-conditioning (e.g., inescapable foot-shock) and conditioning (e.g., conflict /avoidance, discriminative tail-shock) paradigms (e.g., Williams et al, 1971; McCarty et al, 1978; Lawler et al, 1980; Cohen et al, 1984; Randall et al, 1993). When isolated rats were exposed to electrical foot-shock of sufficient

intensity in a non-conditioning paradigm, elevations in several physiological and biochemical parameters, including SBP, were observed, associated with vocalizing and attempting to escape by repeated bursts of running and jumping (Williams and Eichelman, 1971).

A similar experimental procedure utilizing 60 foot-shocks in 10 minutes (2.5 mA of .4 sec duration with an inter-shock interval of 5 sec) was tested on SHR and WKY rats by McCarty and coworkers (McCarty et al, 1978). Increases of HR and SBP in both SHR and WKY were observed in the shock chamber during rest (before electrical foot-shock) compared with home cage levels. While similar to Williams et al's (1971) results, in which HR and behavioral changes during foot-shock stress in both SHR and WKY were reported, further increases in SBP during foot-shock were observed only in WKY, but not in SHR. One explanation might be that SBP measurements were not obtained during the actual delivery of foot-shock, so peak SBP responses were skipped when calculating the stress SBP.

In conclusion, no chronic, sustained hypertension was

reported using non-conditioning electrical shock. However, electrical shock in a conflict/avoidance conditioning paradigm, which will be discussed in next section, was later proved to be very successful in inducing sustained hypertension (e.g., Lawler et al, 1980).

Acute air jet stress was reported to produce tachycardia, elevation of MAP, increase of renal sympathetic nerve activity (RSNA), and decrease in urinary sodium excretion in both SHR and WKY rats (Lundin and Thoren, 1982). Furthermore, changes in urinary sodium excretion and renal nerve activity during stress were greater in SHR than in WKY. The urinary sodium excretion response to air stress was nearly abolished by renal denervation. These results were confirmed by Koepke's study in SHR and WKY animals. Air jet stress increased RSNA 77% and decreased urinary sodium excretion 28% in conscious SHR on a normal diet, as well as producing increases in MAP and HR. No changes were found in WKY (Koepke et al, 1985).

Other physical stimuli tested on animals include cold (Allen et al, 1987) and heat (McMurtry et al, 1981). Acute

elevations in blood pressure and other physiological and biochemical changes were reported in both of the above studies. It may be worth mentioning that ether, as a chemical stimulus, was also given to SHR and Sprague-Dawley rats to test their hormonal responses; unfortunately, HR and blood pressure were not measured during the stress (McMurtry et al, 1981).

Psychosocial Stressors. One problem with studies utilizing physical stimuli is the difficulty in ascribing the effects to "psychological" as compared to "physical" components of the stress. In contrast to physical stimulation, mental and social stressors are non-physical stimuli encountered in the animal's daily life. The central nervous system plays an important role in physiological responses to these stressors. Examples include social isolation (e.g., sensory withdrawal), social challenge, mental tasks, etc.

Maintenance of adequate sensory input to the central nervous system has been shown to be extremely important in the health of cognitive functioning. For example, sensory

deprivation causes depression, hallucinations, and distortions of thought in humans (Scott et al, 1959) and hypertension in rats (Lockett et al, 1973).

Lockett and coworkers (1973) exposed Wistar rats to chronic ambient noise and inbred them for generations. Progressive elevations in blood pressure and HR in response to the chronic auditory stimulation were observed. However, when the inbred Wistar rats were then transferred in pairs to soundproof chambers, the mean arterial pressure (MAP) increased after 2 weeks and reached a plateau of 180 mmHg after 6 weeks of isolation. Lockett and coworkers (1973) proposed that the pituitary-adrenal cortical system is critical for the hypertension caused by sound withdrawal. This conclusion was based on (1) the failure of sound deprivation to produce hypertension in salt-maintained, adrenalectomized, and hypophysectomized rats; and (2) the reinstatement of sound deprivation hypertension if adrenalectomized rats were treated with glucocorticoids.

However, different effects of sensory deprivation on SHR were reported by other authors. Hallback (1975) tested

SHR and normotensive rats housed in socially isolated but not totally sound deprived single cages, with control rats housed 6 in each cage. The isolated SHR, but not the isolated normotensive rats, had significantly lower blood pressure than their non-isolated controls. Reductions in sympathetic arousal and cardiovascular defense reactions were suggested as possible mechanisms for the antihypertensive effect. In another study, young SHR were isolated in a dark room until 12 weeks of age. The rate of development of hypertension was delayed by two weeks compared to control animals (Lais et al, 1974).

One explanation of Hallback's and Lais's results might be that, in their experiments, rats were socially isolated but not as sensory deprived as in Scott's and Lockett's studies. Despite the differences in methodology between the experiments just discussed, the fact that blood pressure responses of SHR to sound deprivation differ from those of normotensive strains suggests that different animal strains may process and integrate sensory information differently.

If social isolation can reduce blood pressure

(Hallback, 1975; Lais et al, 1974), then social stresses such as aggregated housing, territorial stress, and resident attack may cause elevations in blood pressure. This approach has been utilized successfully by several researchers (Alexander 1974; Ely et al, 1983; Henry et al, 1967, 1971 & 1975).

The strongest support for this notion has been provided by Henry in a series of studies using a territorial conflict paradigm. Henry and coworkers (1967) first examined the effects of chronic exposure to four different types of social stress on the development of hypertension in Agouti mice (Henry et al, 1967). The stressors tested were (1) mixing males from different colonies, (2) crowding, (3) exposing males and females to threats from a predator, and (4) creating a situation of territorial conflict using an interconnecting box system. Each of these manipulations produced SBP values of about 160 mmHg in males and 140-150 mmHg in females. Of those stressors, the territorial conflict produced the most substantial increase in SBP.

Henry and coworkers (1975) proposed that "repeated

confrontations serve as an intermittent functional trigger mechanism that leads to repeated arousal of the sympathetic adrenal medullary and the pituitary adrenalcortical neuroendocrine response patterns" (Pg. 156). This hypothesis was based on another study, in which 4 month old Agouti mice were exposed to territorial conflict for 6 months. A significant increase in SBP (170 mmHg), as compared with mice that remained in isolation (126 mmHg), was reported (Henry et al, 1971). Furthermore, stressed mice had heavier adrenal glands, higher levels of plasma norepinephrine and epinephrine, and increased monoamine oxidase and thyroid hormone activity levels.

Later, the reversibility and progression of cardiovascular complications caused by this social conflict were examined (Henry et al, 1975). Sixteen formerly isolated males were placed with 16 normal females in population cages consisting of seven intercommunicating boxes. Animals were removed and studied after periods of interaction ranging from 2 days to 9 months. Following the shorter exposures, blood pressure reverted to normal in a few days. Exposures of 6 months or more were associated with unchanged body

weights and sustained increases in heart weight and blood pressure readings. In addition, there was a significant development of aortic arteriosclerosis and myocardial fibrosis. These changes persisted despite prolonged return to isolation cages.

To most animals, competing for social position, mate, food and water are the most important social-ethological stressors. A territorial stressor was used to examine its influence on blood pressure and brain neurochemistry in SHR (Ely and Weigand, 1983). The SBP was significantly elevated from 2-8 weeks of stress treatment as compared to SHR controls. Norepinephrine levels in the nucleus tractus solitaris and amygdala, and dopamine levels in hippocampus and amygdala, showed significant elevations in the stressed group.

Cardiovascular changes during the "defense reaction" were examined in a resident-intruder aggression test (Viken et al, 1991). The intruder rats responded to resident attack with species-characteristic defensive behavior and increased heart rate (16%), renal resistance (90%), and mesenteric

resistance (200%), and decreased hindquarter resistance (-38%) compared to baseline levels. MAP elevation peaked 12% above baseline; however, the latter effect was not statistically significant. This result might be explained partly by the defeat of intruder animals. Adams and coworkers (1987) reported that repeated defeat of male sensitive/John-Rapp rats was followed by an acute decrement in SBP (30-40 mmHg in magnitude). In contrast, repeated exposures of the same rats to foot-shock were followed by acute increases of SBP (20-40 mmHg) and HR.

Stress in Conditioning Paradigms

The most important advantage of conditioning paradigms is that it affords an opportunity to study the real-time hemodynamic responses in a highly controlled behavior mode. There are two major types of conditioning paradigms, Pavlovian conditioning and operant conditioning.

Pavlovian Conditioning. Pavlovian conditioning, also called classical conditioning, involves the repeated presentation of a conditioned stimulus (CS) (e.g., a tone) with an unconditioned stimulus (UCS) (e.g., tail-shock) to

establish a contingency between them. Presentation of the UCS, usually aversive, will cause acute cardiovascular changes (e.g., elevation of blood pressure) in animals. This response is called the unconditioned response (UR). Over time, the tone will elicit a conditioned response (CR) similar to the UCS. Thus, the CR is considered a psychological response in that the animal is not presented with the aversive event itself, but rather with a signal, or CS, which has been associated with the aversive event (Gormezano et al, 1967). Pavlovian conditioning is the basic, and also the most important, learning method (Rescorla, 1988). So it has been used widely to explore the role of psychological factors in the development of hypertension.

For example, a 10.5 sec, 85-dB, 1-kHz tone (CS) and an electric shock (UCS) were used to examine the effects of Pavlovian conditioning on cardiovascular function in SHR and WKY rats by Hatton et al (1981). The orienting response caused by the tone itself, without UCS pairing, consisted of a HR deceleration and a concomitant increase in blood pressure in both SHR and WKY rats; however, both responses

were exaggerated in SHR. Then the CS and the UCS were paired for 30 trials with an interstimulus interval, or time from CS onset to UCS onset, of 10 sec. The UCS overlapped with the last .5 sec of the CS. The CR in SHR also consisted of a cardiac deceleration coupled with a larger pressor response. The WKY showed a smaller pressor response to the onset of the CS and a significant increase in HR. The HR deceleration response in SHR, but not WKY, was blocked with parasympathetic muscarinic antagonist atropine. This suggests that a stronger parasympathetic reflex reaction was involved in the conditioned response in SHR, while in WKY, less parasympathetic control, or a more dominant sympathetic reaction, was suggested. This has been explained by another study which concluded that the strong pressor response in SHR initiated a strong baroreflex to correct it (Li et al, 1995). In WKY, on the other hand, since the pressor response was much smaller compared to that of SHR, little baroreflex-induced bradycardia resulted.

While traditional classical conditioning theory is based on CS-UCS contiguity and CR-UCS reinforcement (Gormezamo et al, 1967), a new cognitive approach by

Rescorla suggests that contiguity is neither sufficient nor necessary to produce Pavlovian associations (Rescorla, 1988). According to this new theory, the CR is not an identical version of the UCR. What subjects learn is the relationship between the CS and the UCS. Based partially on this new theory, a discriminative conditioning paradigm was developed by Cohen and Randall (1984). a decade ago as a Pavlovian conditioning and learning model to study the effects of behavioral stress on cardiovascular function and the role of the autonomic nervous system. This conditioning paradigm was also utilized in the current study. A 30-sec pulsed tone (positive conditioning stimulus, or CS+) was used as the positive conditioning stimulus, while a non-pulsed tone (negative conditioning stimulus, or CS-) of the same frequency and duration served as the negative conditioning stimulus. The CS+ was followed by a brief, cutaneous electric shock, whereas the CS- was not. This approach has revealed that the canine conditional response consists of reproducible patterns of change in blood pressure and HR. Furthermore, these patterns reveal a clear distinction between CS+ and CS-, and are due to differential activation of the sympathetic and parasympathetic components

of the autonomic nervous system (Randall et al, 1985). These patterns have also been tested in Sprague-Dawley rats using similar procedures. The MAP peaked at 13.6 mmHg above resting level at 1.5 sec after onset of the CS+, compared to 10.0 mmHg on CS-. A greater renal sympathetic nerve response to CS+ than CS- was also reported (Randall et al, 1993).

Properties of the Pulsed and Non-Pulsed Tones. The properties of the tones used in the above conditioning paradigm were tested in a pilot study. Both the pulsed tone and the non-pulsed tone were 15 sec long at 65 dB and 1 kHz though one was pulsed (2 times per sec) and the other was not. In the pilot study, we demonstrated that a non-pulsed tone used as a startle stimulus caused the same MAP response as a pulsed tone. Moreover, it could be as readily conditioned to tail-shock as the pulsed tone (Figure 1). The pilot study used the same instruments and software described in Chapter II of this paper. However, several minor changes in the software controlling the pilot study were made in order to test whether there were differences in responses to the pulsed and non-pulsed tones.

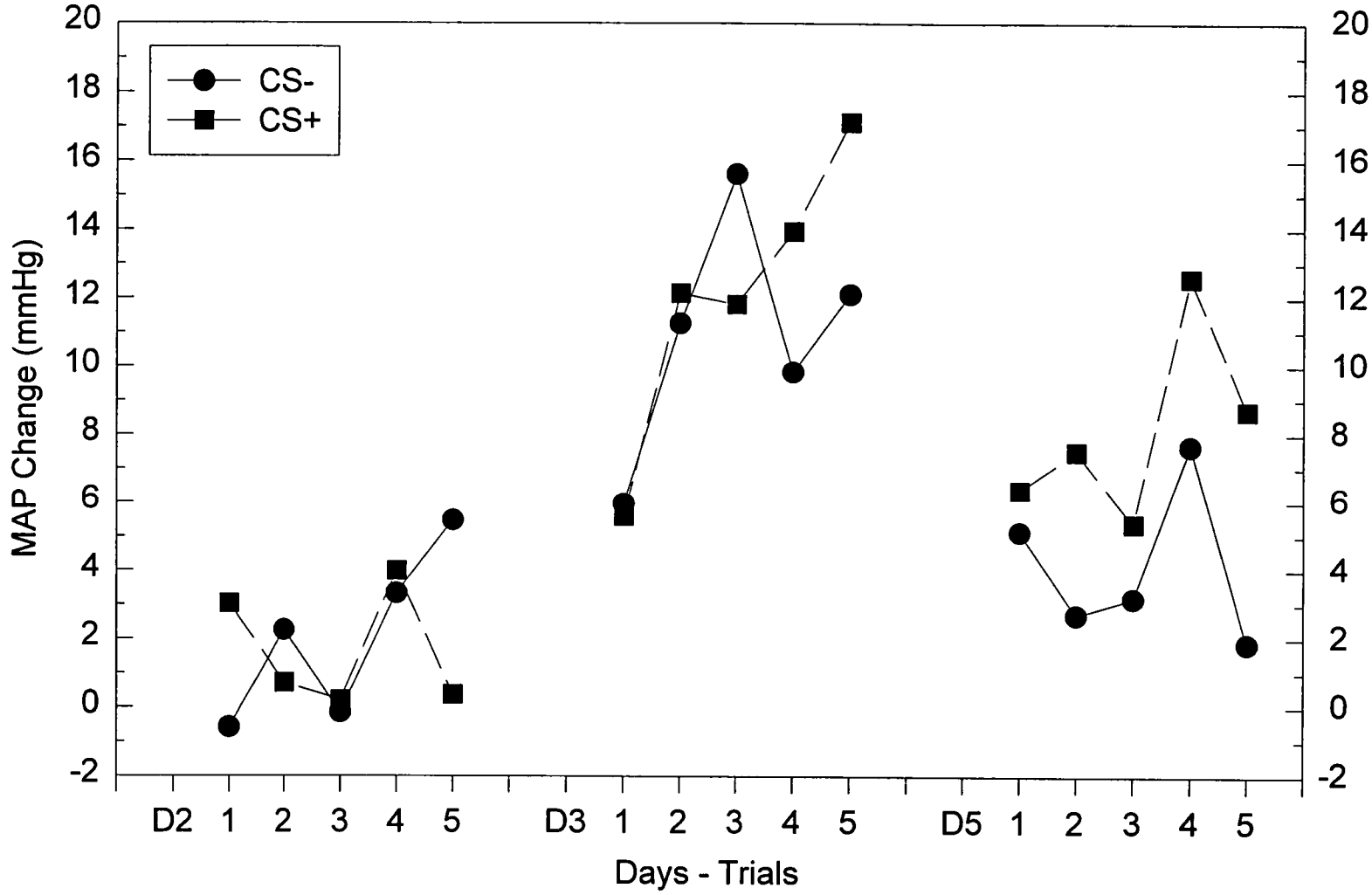

Figure 1. First conditioned response in MAP changes from baseline in conditioning Day 2, 3 and 5 on a Sprague-Dawley rat for 5 CS+ and 5 CS- trials each day with reversed tones. D2=day 2, D3=day three, D5=day five.

Two days after surgery (Day 2 in Figure 1), one Sprague-Dawley rat was given 5 pairs of novel tones (i.e., 5 pulsed tones and 5 non-pulsed tones), neither associated with tail-shock. Figure 1 (left portion) shows that there were no differences in the blood pressure startle response between the pulsed and non-pulsed tones.

Three days after surgery (Day 3 in Figure 1), another 5 pairs of tones were given to the rat. However, this time each non-pulsed tone was associated with a .5 sec, 0.4 mA tail-shock. The rat did not discern the signals for the first pairs of tone. After the first tail-shock, abrupt increases in MAP responses to both tones were evident. During the fourth and fifth pairs of tones, higher MAP responses to the tail-shock associated non-pulsed tone emerged, consistent with the conclusion that rats can easily discriminate between these two trial types.

Five days after surgery (Day 5 in Figure 1), the MAP responses to both trials (pulsed vs. non-pulsed) decreased compared to Day 2. A possible explanation is that the learned predictability (cognition) decreased the level of

anxiety which in turn reduced reactivity. In any event, a discrimination between the MAP responses to the shock associated-tone and to the non-associated-tone was well established. In other words, the animal learned that the non-pulsed tone, in this case, is associated with an inescapable tail-shock. In conclusion, there is no evidence suggesting any differences in psychological properties between the pulsed and non-pulsed tones. They cause comparable MAP startle responses and, as conditioned stimuli, can easily be discriminated.

Operant Conditioning. Pavlovian conditioning has been successful in exploring the physiological, such as cardiovascular and autonomic nervous system, responses to behavioral events; however, permanent hypertension has not been produced with this approach, since the magnitude of the unconditioned response is generally not very large, the periods of conditioning are not long enough so as to constitute a chronic stressor, and the animals tested are usually genetically insensitive to psychological stress. On the other hand, Lawler and coworkers, using operant conditioning paradigms (e.g., conflict avoidance

conditioning) in a series of studies, produced large hemodynamic changes in response to chronic stress, including permanent hypertension (Buchholz et al, 1981; Lawler et al, 1975 & 1980).

Lawler and coworkers (1975) studied the acute hemodynamic responses to an so-called Sidman avoidance (operant conditioning) in 4 dogs for 10 days. This procedure requires the dog to press a lever, in the absence of any external signal, to avoid an electric shock. Each response reset a 20 sec timer. If the 20 sec cycle was allowed to time out before another lever response, an electrical shock was given to the animal. Large individual differences in the hemodynamic response pattern were reported. A distinct cardiovascular pattern emerged in two dogs, characterized by significant increases in arterial blood pressure, resulting from an elevated total peripheral resistance, during pre-avoidance. In contrast, the pressor responses during avoidance were maintained by an increased cardiac output while total peripheral resistance dropped. Both HR and stroke volume contributed to the increased cardiac output.

The other two dogs showed little hemodynamic response to the experimental schedule.

An operant conditioning schedule allows the animal to avoid an aversive shock by pressing a lever or turning a wheel. After some trials, the animal learns the required response and receives less shock. The magnitude of the perceived stress (and, therefore, the cardiovascular response) may decrease as the animal gains complete control over the shock presentation. In order to test this hypothesis, Buchholz et al (1981) employed a shock-shock conflict avoidance paradigm which reduced the ability of animals to control the presentation of the shock. This paradigm required male Long-Evans rats to turn a wheel following the onset of a tone in order to avoid 5 electric shocks to the tail; however, each response then led to the presentation of 1 inescapable electric shock. Consequently, the experimental animals were forced to choose between doing nothing and receiving 5 shocks or giving themselves one shock by turning the wheel and thereby avoiding four additional shocks.

The results of this study demonstrated that shock-shock conflict was indeed more stressful than avoidance alone. The experimental rats showed a significantly increased frequency of readings above 150 mmHg compared to restraint only control animals. Terminal SBP differed significantly between conflict and control groups; however, experimental animals were, at best, only in the "borderline" hypertensive range (146 mmHg). In order to test whether the magnitude of blood pressure was limited by the genetic normality of Long-Evans rats, BHR rats were tested with the same shock-shock conflict stress.

As we mentioned before, the BHR is bred by mating the female SHR with the male WKY, thus representing a moderate genetic predisposition for essential hypertension (Lawler, 1980). As also previously mentioned (p. 24), the BHR has a systolic blood pressure that is intermediate to its parental strains. The BHR response to shock-shock conflict included a chronic elevation in SBP to levels equivalent to those seen in the SHR (185 mmHg), concomitant with myocardial pathology (Lawler et al, 1980). Restraint control animals also showed some elevation of SBP (165 mmHg), but the maturational

controls showed no change in SBP (150 mmHg) during 15 weeks of measurement.

Reactivity to Psychological Stress

Theoretically, we can predict the physiological and behavioral consequence of psychological stress in a group of people, but we remain unsure about its effects on any one individual. This is because the magnitude of an individual's pressor response depends on the sensitivity (reactivity) of the individual to that specific stressor, as well as the intensity and duration of the stressor. The internal determinant of the sensitivity may be the genetic predisposition and the physiological conditions at the time of exposure to the stressor. However, phenomenologically, these may involve any of a number of modifiers, such as family history of hypertension, age, sex, race, physical conditioning, etc. Greater cardiovascular and sympathetic nervous reactivity to various mental stressors have been documented repeatedly in hypertensives and in normotensives at higher risk for developing hypertension (Drummond, 1985; Julius et al, 1991; Light et al, 1983; Santangelo et al, 1989).

Light and coworkers (1983) found that exposure to competitive mental tasks significantly reduced the urinary sodium and volume excreted by young men with one or two hypertensive parents or with borderline hypertension. In this task, the subject who recognized a target stimulus and pressed a telegraph key faster than his competitor won small money incentives. In the high-risk group, the degree of sodium and volume retention was directly related to the magnitude of heart rate (HR) increase during stress, suggesting common mediation by way of the sympathetic nervous system.

Another study involved 16 borderline hypertensive and 10 normotensive young adults. Blood pressure and HR were measured during a pre-test period and during three test periods (mental arithmetic, reaction time, and standing). Increases in SBP were greater in borderline hypertensives than normotensives during all three experimental tasks, while no differences in the number of mental arithmetic mistakes and reaction times were found between groups (Drummond, 1985). Also, higher MAP and forearm vascular resistance were found in borderline hypertensive males than

normotensive males during a mental arithmetic task (Santangelo et al, 1989).

However, whether hyperreactivity is predictive of the future development of hypertension is very controversial (Manuck, 1994). Julius et al (1991) found that hyperreactivity to mental stress was seen more often in the offspring of hypertensive parents, but that such hyperreactivity was unrelated to the future development of hypertension. This may be due to the importance of other environmental determinants, such as dietary salt intake, of blood pressure besides psychological stress.

III. DIETARY SODIUM INTAKE AND HYPERTENSION

Sodium chloride is the major ionic component that determines plasma and extracellular osmolarity and controls blood volume. A change in blood volume will directly influence blood pressure. However, a number of compensatory systems can help the human body to adapt to the alterations (increase or decrease) of salt intake and maintain plasma volume and blood pressure in a narrow range. On the other

hand, the dysfunction of these systems may cause hypertension. Numerous epidemiological surveys and experimental studies support the view that high dietary salt intake can potentiate the development of hypertension, although the actual mechanism(s) remains unknown. The role of dietary salt (sodium chloride) intake as a major environmental factor in the development of hypertension has been tested in this and other laboratories on experimental models. Hypertension was induced by a high salt diet (8% NaCl in chow) in BHR and SHR (Lawler et al, 1987 & 1993), as well as in several other animal models (Mainsail and Drueke, 1992). Several mechanisms have been proposed, such as renal dysfunction (Guyton, 1972), elevated sympathetic activity (Gavras, 1986), and chronic natriuretic hormone release (Kohno et al, 1987; Sagnella et al, 1991).

Role of the Kidney in Salt Induced Hypertension

Dietary salt may influence blood pressure only through an alteration in renal function. Actually, a direct relationship has been documented between renal function and blood pressure in several genetic models of hypertension. For example, when the kidneys from hypertensive rats were

transplanted to their normotensive counterparts, these animals developed chronic high blood pressure (Dahl et al, 1974). Conversely, hypertensive rats receiving kidneys from normotensive animals showed no change in blood pressure. The subsequent hypothesis is that renal dysfunction is required for high salt intake to cause blood pressure elevation. In other words, the renal pressure-natriuresis relation can shift, thereby requiring elevated blood pressure to achieve adequate sodium excretion.

These data are consistent with Guyton's conceptual model (1972; 1992) which assigns a critical role for the kidneys in salt-induced hypertension. According to his schema, renal NaCl excretory defects combined with large sodium intakes exceed excretory capacity. This leads to sodium and water retention and blood volume expansion. Augmented venous return increases cardiac preload, thus reflexively enhancing cardiac output and elevating blood pressure. After the cardiac output has risen to a high level and has initiated the hypertension, the excess blood flow through the tissues then causes progressive constriction of the local arterioles (autoregulation), thus returning the

cardiac output almost to normal, but simultaneously causing a secondary increase in total peripheral resistance. This will eventually cause the development of chronic hypertension. Consistent with this hypothesis, diminished capacities for sodium and water excretion in response to elevated arterial pressure occurs in anesthetized SHR at very early ages (3-5 weeks) before the onset of hypertension. At older ages (12 weeks), when the hypertension is well established, the reduction in pressure natriuresis becomes even more pronounced compared to WKY (Roman, 1987).

Elevated Sympathetic Outflow and Renin-Angiotensin Activity

Excess salt intake may also elevate blood pressure through stimulation of central and peripheral sympathetic activity. Consistent with this possibility, lesions in sympathetic control centers of experimental animals prevent the expression of salt-induced hypertension (Lee et al, 1989). Experiments with BHR indicate that lesion of the anteroventral area of the third ventricle can prevent high salt-induced hypertension in BHR (Sanders et al, 1989a). However, our knowledge about central cardiovascular

regulation is still very limited. The actual mechanism of such an effect is unknown. Gavras (1986) proposed that the normal function of brainstem catecholaminergic neurons is to exert a constant tonic inhibition of sympathetic activity. The Na^+ ion can diminish the affinity of central inhibitory α_2-receptors for their agonists. The disinhibition of the sympathoinhibitory effect of these neurons will eventually increase peripheral sympathetic outflow.

Similarly, salt-induced blood pressure elevations can alter transmitter activity in the anterior hypothalamus of the spontaneously hypertensive rat (Oparil et al, 1988). Excess salt intake may also alter sodium channel activity in the anterior hypothalamus leading to reduced central norepinephrine activity followed by elevated peripheral activity (Chen et al, 1988). Increased sympathetic outflow may increase blood pressure directly by vasoconstricting or by changing kidney function via renal sympathetic nerves. The latter effect includes the activation of the renin-angiotensin-aldosterone system, thus increasing renal tubular sodium reabsorption. We will discuss these factors in detail in subsequent sections.

Role of Atrial Natriuretic Factor

The role of atrial natriuretic factor in salt-sensitive hypertension still remains controversial, in spite of the fact that it has been the focus of many studies in the last decade. Studies show that atrial natriuretic factor has diuretic, natriuretic, vasodilator, sympatholytic, and renin- and aldosterone-suppressing functions (Kuchel et al, 1987). This raises the possibility that hypertension might be a consequence of reduced secretion or action of atrial natriuretic factor. However, most studies have shown that serum levels of atrial natriuretic factor are increased, not decreased, and high salt intake causes a normal or exaggerated rise in plasma atrial natriuretic factor in hypertensive patients (Kohno et al, 1987; Sagnella et al, 1991). These results are so controversial that two seemingly contradictory hypotheses have been proposed.

Blaustein (1985) proposed that excess dietary salt intake may elevate blood pressure through natriuretic hormone, which is an inhibitor of Na^+,K^+-ATPase (Na^+-K^+ pump) in renal tubules and vascular smooth muscle. Normally, this hormone reduces tubular reabsorption of sodium by inhibiting

the Na^+-K^+ pump, leading to natriuresis and diuresis. However, chronic elevations in natriuretic hormone may increase arterial pressure by widespread inhibition of Na^+,K^+-ATPase in vascular smooth muscle, depolarizing the cell membrane, and eventually increasing calcium and sodium influx. This, in turn, increases the excitability and contractility of both cardiac muscle (i.e., cardiac output) and vascular smooth muscle (i.e., peripheral resistance). Increased calcium in sympathetic nerve terminals also enhances norepinephrine release and causes blood pressure elevation. Consistent with this, increased intracellular calcium was found in lymphocytes of salt-sensitive subjects on a high salt diet, but not in those of nonsensitive persons (Oshima et al, 1989).

However, others argue that the increased secretion of atrial natriuretic factor after salt loading might be secondary to volume expansion and stimulation of arterial stretch receptors (Lang et al, 1985). Thus, the increased atrial natriuretic factor may just be an adaptive response to minimize the increase of blood pressure caused by salt loading. Several studies supported the notion that a defect

in atrial natriuretic factor secretion might contribute to the development of hypertension. For example, blockade of endogenous atrial natriuretic factor with specific monoclonal antibodies increases the severity of hypertension in stroke-prone SHR and deoxycorticosterone acetate-salt rats (Itoh et al, 1988). On the other hand, chronic atrial natriuretic factor infusion via osmotic minipumps prevented the blood pressure elevation in response to high salt loading in salt-sensitive SHR but had no effect on blood pressure in rats on normal diet (Jin et al, 1990). Several other hormones may also be involved in the development of salt-induced hypertension. However, none of them has been clearly implicated.

IV. SPECIAL ROLE OF RENAL NERVE ACTIVITY IN HYPERTENSION

Role of Efferent Renal Nerve Activity

Most of the evidence about renal nerve effects on the kidney comes from renal denervation and stimulation studies. Complete renal denervation prevents or delays the onset of chronic stress- (Lawler et al, 1989) or salt-induced

(Winternitz et al, 1980) hypertension in some animal models; conversely, renal nerve stimulation increases renin secretion, and decreases sodium and water excretion, renal blood flow and glomerular filtration rate (DiBona, 1985; Wyss et al, 1992).

Relative to its size, the kidney has an extraordinarily rich innervation. Electron-microscopic (Barajas, 1964; Barajas et al, 1992) and histochemical fluorescence (Kuyper et al, 1979) methods have been used to demonstrate a predominantly noradrenergic innervation of the afferent and efferent arterioles, the juxtaglomerular apparatus, the proximal and distal tubules, and the thick ascending limb of the loop of Henle. Both afferent and efferent renal nerves play important roles in the regulation of plasma volume, sodium concentration, and blood pressure. Bilateral renal denervation in SHR delays the onset and slows the development of hypertension and increases urinary sodium excretion (Winternitz et al, 1980).

Most renal efferent sympathetic innervation originates from ipsilateral paravertebral ganglia from thoracic segment

11 to lumbar segment 3. These fibers are predominantly unmyelinated (slow conducting) noradrenergic fibers. There is little evidence of cholinergic innervation of glomerular arterioles (Barajas, 1992).

The major effects of efferent renal nerve activity on kidney function, and a possible role in hypertension, are: (1) renal vasoconstriction, (2) enhanced tubular reabsorption of sodium and water (antinatriuresis and antidiuresis, respectively), and (3) increased renin secretion. Different intensities of renal nerve activity affect these functions differently. This is illustrated by a study with renal nerve stimulation at different frequencies (DiBona, 1985). Using electrical stimulation at .25 Hz, renin secretion rate increased without changing urinary sodium excretion, glomerular filtration rate, or renal blood flow. At 1.00 Hz, renin secretion rate increased and urinary sodium excretion decreased without changing glomerular filtration rate or renal blood flow. When the stimulating frequency was increased to >2.0 Hz, renin secretion rate increased and urinary sodium decreased as before. However, this higher frequency stimulation also reduced glomerular

filtration rate and renal blood flow.

Effect on Renal Vasoconstriction. Renal blood flow is not affected by a 60% increase in efferent renal nerve activity produced by carotid sinus baroreflex activation. Withdrawal of basal efferent renal nerve activity by renal denervation does not affect renal blood flow (Koepke et al, 1985). This may account for the failure of psychological stress to produce significant blood pressure increases in Dahl rats and some other animal models (Friedman et al, 1975; Adams et al, 1987). However, greater increases in efferent nerve activity to about 500%, as demonstrated by substantial environmental stress in BHR and other animals, produce profound renal vasoconstriction (DiBona, 1982). Thus, renal blood flow is maintained relatively constant unless sympathetic nervous system activity increases substantially.

Another interesting and important phenomenon is that glomerular filtration rate is maintained constant even if renal blood flow drops 15% or more, or when MAP changes from 75 to 160 mmHg. The mechanism for this is that, when

efferent renal nerve activity increases, it mainly causes constriction of afferent glomerular arterioles rather than efferent arterioles. Renal sympathetic activity at low intensity (e.g., a stimulation of .5 Hz) can increase renin secretion, which in turn activates angiotensin II, which primarily causes constriction of the efferent glomerular arterioles, rather than the afferent glomerular arterioles. The efferent glomerular arteriole constricts slightly more than, or the same as, the afferent arterioles, so that the glomerular filtration pressure and the glomerular filtration rate do not decrease, or may even increase slightly, at low intensity (or low frequency) renal nerve stimulation.

Effects on Renal Tubules. As mentioned previously, stimulating the renal nerve at 1.0 Hz can increase sodium reabsorption by the proximal, convoluted distal tubules and the thick ascending limb of the loop of Henle, mediated by alpha-1 adrenergic receptors. The denervated kidney of conscious rats exhibits an increase in urinary sodium and water excretion unaccounted for by changes in glomerular filtration rate or renal blood flow (DiBona, 1982). Wyss (1992) argues that the natriuresis of denervation is

dependent on a decrease in efferent renal nerve activity, whereas the diuresis is dependent on a decrease in plasma antidiuretic hormone. Efferent renal nerve activity also increases renin secretion which in turn activates angiotensin II and aldosterone. Aldosterone increases Na^+-K^+ ATPase pump synthesis and activity, increasing Na^+ reabsorption and K^+ secretion in both the distal tubule and cortical and cortical-medullary collecting ducts.

Effects on Renin Secretion. Three major mechanisms are involved in renin secretion by juxtaglomerular granular cells: the renal vascular baroreceptor, the tubular macula densa receptor (located between the distal tubule and the thick ascending limb of the loop of Henle), and renal sympathetic tone (neural and hormonal). Although it is difficult to isolate and identify the effect of efferent renal nerve activity on renin secretion, evidence suggests that stimulation at a low frequency (.5 Hz) can increase renin secretion without influencing Na+ secretion, glomerular filtration rate, or renal blood flow, as mentioned previously (DiBona, 1985).

Kopp (1992) recently suggested another role for efferent renal nerve activity: it can facilitate afferent renal nerve activity. Single unit recording suggested that stimulating the efferent renal nerve enhanced chemoreceptive or mechanoreceptive afferent renal nerve activity. An efferent-afferent renal nerve facilitatory mechanism was proposed. This mechanism may enhance the sensitivity of the renal receptors.

While the evidence discussed above suggests a potential role for efferent renal nerve activity in the development of hypertension, some evidence is contradictory. In a recent review, Wyss (1992) concluded that the renal nerves do not contribute to dietary NaCl-exacerbated hypertension in SHR, dietary NaCl-induced hypertension in Dahl-NaCl sensitive rats, or the chronic hypertensive effects of cyclosporine-A therapy in rats. However, this does not undermine the potential role of renal nerve activity on renal function and hypertension in other animal models. Although it is difficult to reconcile the discrepancies, given our current state of knowledge, there are probably different mechanisms involved in different animal models of hypertension. Another

possibility is that there is a lack of comparability among different studies due to differences in animal models, procedures, techniques or instrumentation.

Role of Afferent Renal Nerve Activity

Renal afferent nerves are predominantly unmyelinated (slow conducting) adrenergic fibers. These fibers enter the spinal cord through dorsal root ganglia from thoracic segment 6 to lumbar segment 2. Afferent fibers are associated with arterial and venous vasculature, corticomedullary connective tissue, and the pelvic region. Approximately 8% of the renal afferent fibers project directly to the medulla, forming the monosynaptic, myelinated (fast-conducting) renobulbar pathway. This is important in rapidly signaling the medulla concerning renal mechanical and chemical changes. This, in turn, leads to rapid, transient modulation in sympathetic nervous system function.

Renal Mechanoreceptors. An inverse relationship was found between arterial blood pressure and renal arterial baroreceptor activity from kidneys perfused at a constant

flow rate with Ringer's solution (Niijima, 1971). This is especially important because it is different from the baroreceptors in the carotid bodies and aortic arch. That is, renal baroreceptors are more responsive to hypotension (low arterial pressure), while other baroreceptors are more responsive to increases in blood pressure. Thus, renal baroreceptors are more adapted to be sensitive to ischemia, while aortic and carotid baroreceptors are adapted to be sensitive to high blood pressure.

Renal venous mechanoreceptors, on the other hand, are sensitive to renal venous congestion (increase in pressure). Activation of renal afferent nerves by means of renal venous congestion results in a reflex inhibition of efferent nerve activity to the contralateral kidney and to the cardiopulmonary region in dog (Kopp, 1984). This is the so-called renorenal reflex.

Renal Chemoreceptors. Two kinds of chemoreceptors, type 1 and type 2, have been identified in renal arterioles, veins, and tubules in rats (Recordati, 1980). Both type 1 and type 2 receptors are sensitive to ischemia. However,

type 2 may be more important in pressure control since (1) type 2 receptors have a basal discharge under normal physiological conditions, while type 1 receptors do not; (2) type 2 receptors are the principal source of ongoing activity observed in multiunit recordings of afferent renal nerve; (3) type 2 receptors are also sensitive to tubular K^+ and Na^+ concentrations; and (4) fewer type 2 receptors were reported in old SHR, while WKY rats have only the type 2 receptors with no type 1 receptors.

Despite the recent progress described above, however, direct evidence for the role of the afferent renal nerve in hypertension is still lacking. Total renal denervation (lesion of both the afferent and efferent renal nerves) significantly attenuated the normal rise in blood pressure in SHR (Winternitz et al, 1980). Left dorsal rhizotomy attenuated renovascular hypertension in one-kidney, one-clip Sprague-Dawley rats (with right kidney removed and left renal artery clipped) (Wyss et al, 1986). However, unilateral dorsal rhizotomy (lesion of thoracic segment 6 to lumbar segment 2 afferent spinal nerves only) had no effect in SHR (Opril et al, 1987). Nevertheless, these latter

findings do not necessarily rule out a role for the afferent renal nerve in the pathogenesis of hypertension, since it is possible that one intact afferent renal nerve might be sufficient for signaling changes of pressure and ion concentration in the kidneys.

In conclusion, there is much evidence supporting a role of both efferent and afferent renal nerve involvement in the development of hypertension in some animal models, yet many questions remain to be answered. Studies of the interaction between renal nerve activity and cardiovascular function in conscious animals are especially needed.

V. INTERACTIONS AMONG STRESS, DIETARY SODIUM INTAKE AND GENETICS

Genetic predispositions, dietary sodium intake, and reactivity to daily stress events are thought by many researchers to be the major interacting factors in the development of hypertension (Guyton, 1972 & 1992; Page, 1963). Although the search for a single fundamental abnormality responsible for the hemodynamic cascade toward

sustained hypertension continues to attract the imagination and energies of numerous investigators, there may be no such single defect. In view of the multiple factors involved in the control of blood pressure, the concept of a multifaceted mosaic, introduced by Page (1963), has been supported by many studies.

For example, a high salt diet may either enhance sympathetic nervous system reactivity to environmental stress and/or diminish the ability to recover from the stress state. Environmental stress may be translated into increased sympathetic nervous system activity via the limbic-hypothalamic-bulbar autonomic centers. The strongest evidence has probably come from the series of studies done with BHR. The development of hypertension in rats with a moderate genetic predisposition is accelerated by high dietary NaCl intake alone (Lawler et al, 1987) or by environmental stress alone (Lawler et al, 1981). Furthermore, the combination of genetic predispositions, high dietary NaCl intake, and environmental stress results in more severe hypertension than any factor alone (Sanders et al, 1988). These animals fed a high salt diet show an

antinatriuretic response to stress, while BHR on normal salt and WKY on normal or high salt do not (Sanders et al, 1988; Lawler et al, 1991). Bilateral renal denervation can prevent stress-induced hypertension in the BHR, but only if it is done during a critical phase (before the elevation in blood pressure plateaus). This suggests an essential role for the renal nerve in the early weeks of exposure to stress (Lawler et al, 1989).

Previous evidence suggests that hypertension provoked by exposure to stress may be mediated in large part by an exaggerated responsiveness of the sympathetic nervous system (Folkow, 1993). On the other hand, increases in arterial pressure resulting from maintenance on a high salt diet are widely thought to be intimately related to volume expansion (Guyton, 1992). As mentioned previously, experiments with the BHR indicate that lesion of the anteroventral area of the third ventricle can prevent both high salt-induced (Sanders et al, 1989a) and stress-induced (Sanders et al, 1989b) hypertension, which suggests that this forebrain area is a common neural structure underlying both forms of disorder. Furthermore, studies suggest that renal

sympathetic nervous activity and kidney function play a very important role in both salt- and stress-induced hypertension (Guyton, 1992; Osborn et al, 1991; Sripairojthikoon et al, 1989; Katholi et al, 1983; Diz et al, 1982).

It is important that our approach be as relevant to the etiology of human hypertension as possible. In fact, the interactions among high salt diet, stress and parental history have been reported in humans (Falkner et al, 1981). The cardiovascular response to mental stress was evaluated before and after salt loading in adolescents with or without a family history of essential hypertension. The effects of salt loading on the family history positive group was to increase significantly the stress-induced systolic and diastolic pressure, while the HR response decreased. Salt loading resulted in no change in cardiovascular response to stress in the family history negative group.

VI. STATEMENT OF THE RATIONALE AND PURPOSE

As mentioned before, although the complete mechanisms of essential hypertension are still unclear, the involvement

of genetic predispositions, dietary salt intake, and psychological stress are quite evident. It is also clear that the renal nerves and kidneys play a central role in hypertension. While studies of high salt diet, exercise, and stress effects on cardiovascular and renal function, as well as plasma catecholamines and central neurotransmitter levels, in borderline hypertensive rats (BHR) and other animal models have been reported, direct measurement of renal nerve activity has rarely been documented in any conscious animal model.

It seems clear that hope for continued progress in the treatment/prevention of many cardiovascular diseases, especially hypertension, lies in elucidating the interactive effects of converging "risk factors." A careful, quantitative test of these interactions will yield valuable new insights into the etiology - and eventually the prevention - of hypertension. Practical and ethical considerations dictate that such experiments be conducted in laboratory animals whose genetic backgrounds are well characterized. Integrative studies monitoring renal nerve activity and cardiovascular function simultaneously in

conscious, behaviorally-controlled, animals are especially important. This represents part of the rationale for this study.

In this dissertation study, we investigated the differences in patterns of cardiovascular responses and RSNA among SHR, BHR and WKY rats in two experiments using a conscious animal preparation which permitted (1) reliable recording of RSNA; (2) a highly controlled behavioral stress; (3) manipulation of dietary salt; and (4) selection of subjects, i.e., different animal strains, susceptible or resistant to hypertension. In the first experiment, 36 age-matched SHR, BHR and WKY rats (12 rats in each strain) on normal salt diet were used to test the differences in renal nerve and cardiovascular responses to classical aversive conditioning. In the second experiment, 24 BHR were evenly divided into two age-matched groups, half on high salt (8% NaCl in chow) for 8 weeks and the other half on normal salt diet. The effects of high salt diet on cardiovascular and RSNA and their responses to classical aversive conditioning were then examined. This study sought to achieve the following goals:

1. To determine if there are different patterns of renal sympathetic activity and cardiovascular responses to classical discriminative conditioning among different animal strains, i.e., SHR, BHR and WKY.

2. To examine the renal sympathetic nerve and cardiovascular pressor effects of intermediate-term high salt load (8 weeks) in BHR with a moderate genetic hypertensive predisposition in comparison with normal diet BHR.

3. To examine the pattern of cardiovascular and renal sympathetic nerve responses to a tone associated with cutaneous shock compared to a tone not associated with shock.

4. To examine the interaction between high salt diet and aversive discriminative conditioning on cardiovascular and RSNA.

CHAPTER II

METHODS AND MATERIALS

I. SUBJECTS

Twelve male SHR, 12 male WKY and 24 male BHR 14 weeks of age were utilized in this study. All experiments were conducted using the facilities of the Cardiovascular Laboratory, Department of Physiology and Biophysics, University of Kentucky College of Medicine, Lexington Kentucky. All rats were purchased from Taconic Farms (Germantown, New York) at 4 weeks of age. The animals were housed according to strain, 3 in each cage, until they were 12 weeks of age. At this point, the animals were operated on and individually housed until the end of the experiment.

II. APPARATUS

Home Cages

The rats were housed according to strain, 3 in each cage, in standard plastic laboratory cages under a 12 hour/12 hour light/dark cycle in a temperature-controlled

room. All animals had access to water ad libitum, and appropriate rat chow. All SHR, WKY and half (N=12) of the BHR animals were maintained on normal salt (NaCl .8%) laboratory rat chow for the whole experimental period. The other half (N=12) of the BHR were maintained on high salt (NaCl 8%) chow beginning 8 weeks before measurement and termination. Before the high salt manipulation, these BHR were maintained on normal salt rat chow.

Recovery Cages

The surgical procedure will be described in subsequent section. After surgery, each rat was individually housed in a metal net cage until they were terminated. The cage was 30 cm x 30 cm x 35 cm in size and was placed on a plastic platter with soft bedding on it. Animals had access to the same tap water ad libitum and chow as in their home cages. The top wall of the recovery cage was made of plexiglas with an opening (3 cm x 20 cm in size) in the middle. This opening allowed a flexible wire tether (stainless steel spring), which protected catheters and electrodes exiting the rat, to slide back and forth as the rat moved in the cage. The opening prevented the spring from twisting.

Conditioning Instrumentation

A soft, conical terry-cloth sock was used as a mild restraint during conditioning and measurement. Rats had complete freedom of movement in the sock. However, once accustomed to it, rats typically remained quiet, requiring only occasional re-introduction. This device made it possible to record cardiovascular and renal sympathetic nervous system changes during classical conditioning in conscious animals with minimal behavioral restraint.

A 486-based microcomputer with interface and software was used to control the onset and duration of a pulsed (CS+) and a non-pulsed (CS-) tones as well as shocks, for the discriminative conditioning paradigm. Each tone was 15 sec long at 65 dB and 1 kHz. The tones were controlled by the computer so as to be presented in pseudo-random pairs (e.g., CS-, CS+, CS+, CS-). A two-pole shocker (Coulbourn Instruments Company) was controlled by the computer and could deliver current through two electrodes secured on opposite sides of the subject's tail. The intensity of the shock was adjusted individually to the lowest value (usually .3-.4 mA) which caused the rat to flinch; shock intensity

never exceeded .5 mA. Shock duration was fixed at .5 sec for each CS+ trial.

Cardiovascular and Renal Sympathetic Nerve Recording Equipment

MAP and HR were measured directly by a chronically implanted arterial catheter (Teflon tubing, ID=.012 in; Small Parts, Inc.) attached to a blood pressure transducer (Cobe model CDX-III). The transducer was connected to a low-level D.C. Grass preamplifier (Model 7P1F) and driver amplifier (Model 7DAF) in a Grass 7 channel polygraph (Model 7H 25-60). HR was obtained from the pulsatile arterial pressure signal via a Grass tachograph preamplifier (Model 7P6C) and driver amplifier (Model 7DAF). The MAP and HR signals were also fed into an analog to digital converter and a 486-based microcomputer. Software for controlling the conditioning session and recording physiological responses was stored on the hard drive of the computer, while the physiological data were digitalized and stored on an optical disk drive (capacity 128 MB/disk) for subsequent analysis.

RSNA was measured from each chronically instrumented

rat using two Teflon-coated gold wires (A-M Systems, Inc.) glued on a renal nerve. Each gold wire was about 10 cm in length. The distal ends of those gold wires were soldered to the ends of two regular electrodes. Each of the regular electrodes were about 50 cm long. The electrodes were connected to a Grass 5-1,000 x 1,000 amplifier (Model P511K). The signal was fed into a storage oscilloscope (Tektronix Model 5111) for monitoring and into the computer through the analog/digital converter. The digitalized data were saved in the computer RAM simultaneously and saved on the optical disk after each trial. Data for each trial were saved in an individual file. Each file was 1.2 MB in length. A typical experiment produced 10 such files (10 trials) for a total of 12 MB of data per rat.

III. PROCEDURE

General Description

We divided the study into two experiments. In experiment 1, WKY (N=12) and SHR (N=12) were studied. In experiment 2, we studied BHR on a normal (N=12) or high salt (N=12) diet. The data from normal diet BHR will be used to

compared with SHR and WKY for strain differences, as well as with high salt BHR group for the effects of high salt intake. All SHR and WKY rats, as well as half (N=12) of the BHR, were given a normal diet. The other half (N=12) of the BHR were placed on a high salt diet (8% NaCl in chow) for 8 weeks prior to measurement. Animals were assigned to 4 groups as shown in Table 1.

Table 1. Group Assignment for Strain, Diet, and Age of Measurement

Group	Strain	Diet	Age at Measurement
1	WKY	normal	14 weeks old
2	SHR	normal	14 weeks old
3	BHR	normal	14 weeks old
4	BHR	8% NaCl	14 weeks old

The starting dates for salt diet were staggered for individual animals, so that all high salt diet animals had been fed with 8% NaCl chow for 8 weeks prior to measurement and termination. By keeping terminal age constant, we

assured roughly equal body size in all groups, which made interpretation of cardiovascular and renal nerve activity data more comparable among groups.

As the conditioning, surgery and measurements began, we processed four rats each week. The rats were given one week of habituation and conditioning prior to surgery and measurements. Starting with the second week, we habituated and conditioned another 4 rats for surgeries and measurements for the following week, while performing surgeries and measurements for 4 rats that had been conditioned during the prior week. In other words, starting with the second week, 4 rats were habituated and conditioned every week and also 4 rats were operated and measured in the same week. HR, MAP and RSNA were continuously recorded and stored in the computer during the 10 measurement trials. An individual file (1.2 MB in length) was created for each trial.

Discriminative Conditioning

The animals were habituated for 1 hour daily to handling and mild restraint in a soft, conical terry-cloth

sock for 2 days. No shocks or tones were given during these two days. The purpose of this procedure was to allow the animals to get habituated to the sock device.

On the third day, each rat was exposed to 5 trials of pulsed (pulsed twice per sec) tone (eventually to become the CS+) and 5 trials of an otherwise identical, but continuously-sounded tone (eventually to become a non-reinforced CS-), both 15 sec in duration at 65 dB and 1 kHz. The tones were presented in pseudo-random pairs (e.g., CS-, CS+, CS+, CS-). To allow equal familiarization with the tones, none of the first 4 sets of tone were paired with shock. However, the fifth (final) CS+ tone on this day was terminated with a .5 sec tail-shock.

On the fourth and fifth days, each rat was conditioned with 5 trials of each tone (CS+ and CS-) daily. This time, however, each CS+ trial was followed by an electrical shock. The intensity of the shock was individually adjusted to the lowest value (usually .3-.4 mA) which caused the rat to flinch; shock intensity never exceeded .5 mA. The CS- was never associated with shock. A minimum of 5 minutes elapsed

between the presentation of tones (trials). This assured that the animal's blood pressure, HR rate and RSNA had returned to per-trial levels. The same behavioral manipulations were used during the measurement period except that the sequence of CS+ or CS- trials was randomly arranged. Rats were removed from the sock at the end of each daily session and returned to their home cages. The investigator remained in the laboratory with the rat during these and all later experiments to control the tests and observe the animals, but noise and direct interaction with the subjects were minimized.

Catheterization

All surgical procedures were conducted in a room using "sterile" procedures. Rats were anesthetized with sodium pentobarbital (65 mg/Kg, IP) in preparation for the implantation of the arterial catheter and renal nerve electrodes. Surgery was conducted using sterilized instruments and implantation techniques. Infection has not been a problem using this approach.

An arterial catheter, made of Teflon, with an internal

diameter of .012 inch (Small Parts, Inc., Miami Lakes, FL), was prepared by filling with 20 u/ml heparinized saline to prevent blood from clotting inside the tubing. The femoral artery was exposed and the catheter was inserted 5-6 cm deep into the vessel and tied. After surgery or measurement, each catheter was flushed with saline and refilled with 50 u/ml heparinized saline daily till the termination of the animal. The tip of the arterial catheter remained in the upper abdominal aorta. The signal characteristically showed clearly defined systolic and diastolic pressures. The distal end of the catheter was tunnelled under the skin, then joined with the electrodes, all of which exited at the nape of the neck. A flexible tether, consisting of a stainless steel spring, allowed these leads to be securely fastened to the physiological monitoring equipment.

Renal Nerve Electrode Implantation

Following catheterization, a 6 cm long incision was made along the left Harrison's groove (immediately under the edge of the ribs). The abdominal muscle and connective tissue were separated and pushed apart with a surgical retractor. Q-tips and saline-soaked gauze were used to push

the left kidney slightly forward and expose the posterior side of the kidney and renal vessels. Under a dissecting microscope, the left renal artery was exposed and a sympathetic nerve coursing over the aorta toward the kidney was identified. A small section of this nerve, usually at the cephalad angle formed by the renal artery and aorta, was dissected free from connective tissue and placed on fine, closely spaced (.4-.8 mm), bipolar gold electrodes (A-M Systems Inc., Seattle, WA). The exposed nerve and electrodes were encased in silicon gel (Wacker Chmie Gmbh, Munich). The distal ends of the wires, soldered to the end of the electrodes, were joined with the arterial catheter and tunnelled under the skin, exiting at the nape of the neck. Finally, the animal was returned to its recovery cage after surgery.

Blood Pressure and Heart Rate Recording

After a 48 hour recovery period from the surgery, HR, MAP and RSNA were measured during ten conditioning trials (5 CS+ and 5 CS- trials each). The behavioral manipulations during measurements were the same as during training, except the sequence of CS+ or CS- trials was randomly determined.

After calibration against a mercury column, HR and MAP, as well as RSNA, were continuously recorded during the 10 trials, after which the rat was returned to its home cage. These measurements were repeated the next day in case the quality of the signal was not sufficient during the first measurement. We were usually able to record data for 2-3 days, although in some animals either the arterial pressure or nerve recording failed after only one day.

Renal Sympathetic Nerve Activity Recording

The rat was set on a experimental table inside of an electrically-shielded, animal isolation unit. This device reduced the electrical-magnetic noise to a minimum. The electrical signal from the renal sympathetic nerve was amplified (50,000 times) and bandpass-filtered between .3-3 KHz using a Grass P511 Differential Amplifier and a Tekronix 5111 oscilloscope display.

Each conditioning and measuring trial lasted 30 sec with an intertrial interval of at least 5 minutes. The RSNA data, as well as MAP and HR data, were digitally sampled at 10,000 Hz using an analog/digital converter and a 486-based

microcomputer. Sampling began 9 sec before presentation of the 15 sec CS+ or CS-tone and continued until 6 sec after the termination of the tone, or 30 sec of total recording per trial. These unabridged digital files were automatically archived to the computer RAM during the trial, and then stored on optical disk after each trial.

IV. COMPUTER DATA ACQUISITION AND ANALYSIS

Data Acquisition

The RSNA, HR and MAP data were initially sampled at a very high frequency of 10,000 Hz to assure maximum accuracy. All of the data were stored on about twenty, 128 MB each, optical diskettes. However, these files were too large and had to be compressed before they could be plotted on a graph and analyzed further. The compression process for HR and MAP data was slightly different from that for RSNA.

Data Analysis

In order to compress the HR and MAP data, we first reduced the blood pressure data to a 1,000 sample per sec signal (from the initial 10,000 sample per sec) by saving

every 10th point and skipping the rest. We then calculated HR and MAP for each pulse based on the 1,000 sample per sec signal. At this point, the HR and MAP data were separated and saved in different subfiles. Finally, the calculated HR and MAP signals were averaged over every 10 points to produce 100 sample per sec signals. Thus, each of the final HR and MAP subfiles has 100 data points per sec, or a total of 3,000 data points per trial (30 sec).

For RSNA, the initial rapid sample signal (10,000 Hz) were full-wave rectified and averaged over every 100 points to produce a 100 sample per sec signal, or a total of 3,000 points per trial (30 sec). This process thus retained cumulative information from the initial signal. The resulting data were stored in compressed form as one of the three subfiles of the trial. The compressed files, in turn, were transferred on to floppy diskettes for subsequent analysis at the University of Tennessee, Knoxville, using a 486-based microcomputer.

As a result of this data compression, while data for each trial were still stored in an individual file, the

final "compressed" files included three subfiles (i.e., RSNA, HR and MAP), each of which contained 3,000 individual data points covering a total of 30 sec per trial (1 sample every .01 sec). A graph using all of these compressed data was plotted from each individual file. Since the tone onset was at the 9 sec mark and lasted for 15 sec, the first 9 sec of the 30 sec recording was the resting control period (sec 0.00-8.99), while the following 15 sec was the tone on period (sec 9.00-23.99), and the last 6 sec was the recovery period (sec 24.00-30.00).

As mentioned above, we measured each rat in 5 CS+ and 5 CS- trials during the day. Since there were variations among different CS+ (or CS-) trials caused by many controllable or uncontrollable factors (e.g., unexpected animal movement), an averaging of all five CS+ (or CS-) could better represent the "true" responses of the animal during the same measurement of the day. In order to do that, each of the five individual CS+ (or CS-) data files conducted over that day were combined for each rat to yield a "high resolution" analysis file by ensemble averaging analysis. This was called an ensembled file. Each of the ensembled files

represents a rat during either CS+ or CS- trials. That is, for the final results, each rat had two ensembled files, one ensembled CS+ file and another ensembled CS- file.

Only one ensembled CS+ file and one ensembled CS- file were calculated and used from each animal in order to assure that each animal was treated as one observation only. All of the data were calculated from these ensembled files of individual animals. In other words, these files were analyzed for each rat to determine the various parameters such as the resting and the stress values of RSNA, MAP and HR responses.

Statistics

To compare the differences among the three strains, anova tests were carried out. Independent t-tests were also used to make between group comparisons, such as that between different strains or between high salt and normal salt BHR. Since all rats received both CS+ and CS- trials, paired t-tests were used to test the differences between the two trial types within each group. Correlation analyses were carried out on such measures as the RSNA and MAP to explore

their relationship during some specific periods. A minimum level of significance (viz., $p<.05$) was applied to all statistical tests.

In order to view the overall graph of each group during either type of trial (CS+ or CS-), two high resolution files (one for CS+ and another for CS-) were produced for each group by averaging across all animals in the same group. These were called "composite" files. Similar to the ensembled files, each "composite" file also contained 3,000 data points covering 30 sec of trial. However, the "composite" file was made of all CS+ or CS- trials of all animals in a group, instead of being made of 5 CS+ or CS- trials of one animal as in an ensembled file. The purpose of making a "composite" file was to give us an overview of each group's data. But no statistics were calculated from the "composite" graphs because it represented a group average rather than individual observations.

CHAPTER III

RESULTS

A total of 48 male rats were involved in two experiments. Following a general description of the time sequence of the conditioning response in all rats, the subsequent two sections will deal specifically with each experiment. In both sections, the differences between groups and between trial types on all RSNA, HR and MAP measurements will be illustrated. In the first part, the results from the three animal strains (i.e., WKY, BHR, and SHR) with different genetic predispositions to hypertension will be described. In the second part, the results from BHR on normal vs. high salt (NaCl) diet will be compared to examine the influence of high sodium intake on RSNA, HR, and MAP. To show an overview of the responses of each group during either trial type (CS+ or CS-), the "composite" files for each group and trial type were calculated by averaging across all animals in the same group (method described in chapter II). These files were plotted as Figures A-1 to A-8 and are included in appendix A. As mentioned previously, all

data were quantified based on individual files of each animal during each type of trial rather than the "composite" files. All measurements are expressed as mean and standard error (mean±s.e.) unless otherwise specified.

I. TIME SEQUENCE OF CONDITIONING

In general, all animals showed a similar conditioned response pattern for RSNA, HR and MAP, though the magnitude of these measurements was different between groups and trial types. In other words, all animals shared the same time sequence of responses and direction (increase or decrease) of change in all measurements. Figure 2 depicts a typical pattern for RSNA, HR, and MAP in one CS+ trial. The time sequence of the these responses is illustrated in Table 2.

All RSNA, HR, and MAP measurements were relatively stable during the first 9 sec of baseline (resting level). A series of changes were observed during the 15 sec tone period (sec 9.00-23.99). The onset of a pulsed (CS+) or a non-pulsed (CS-) tone at the 9 sec point caused a sudden burst of RSNA (referred to as SB in Figure 2) with the same

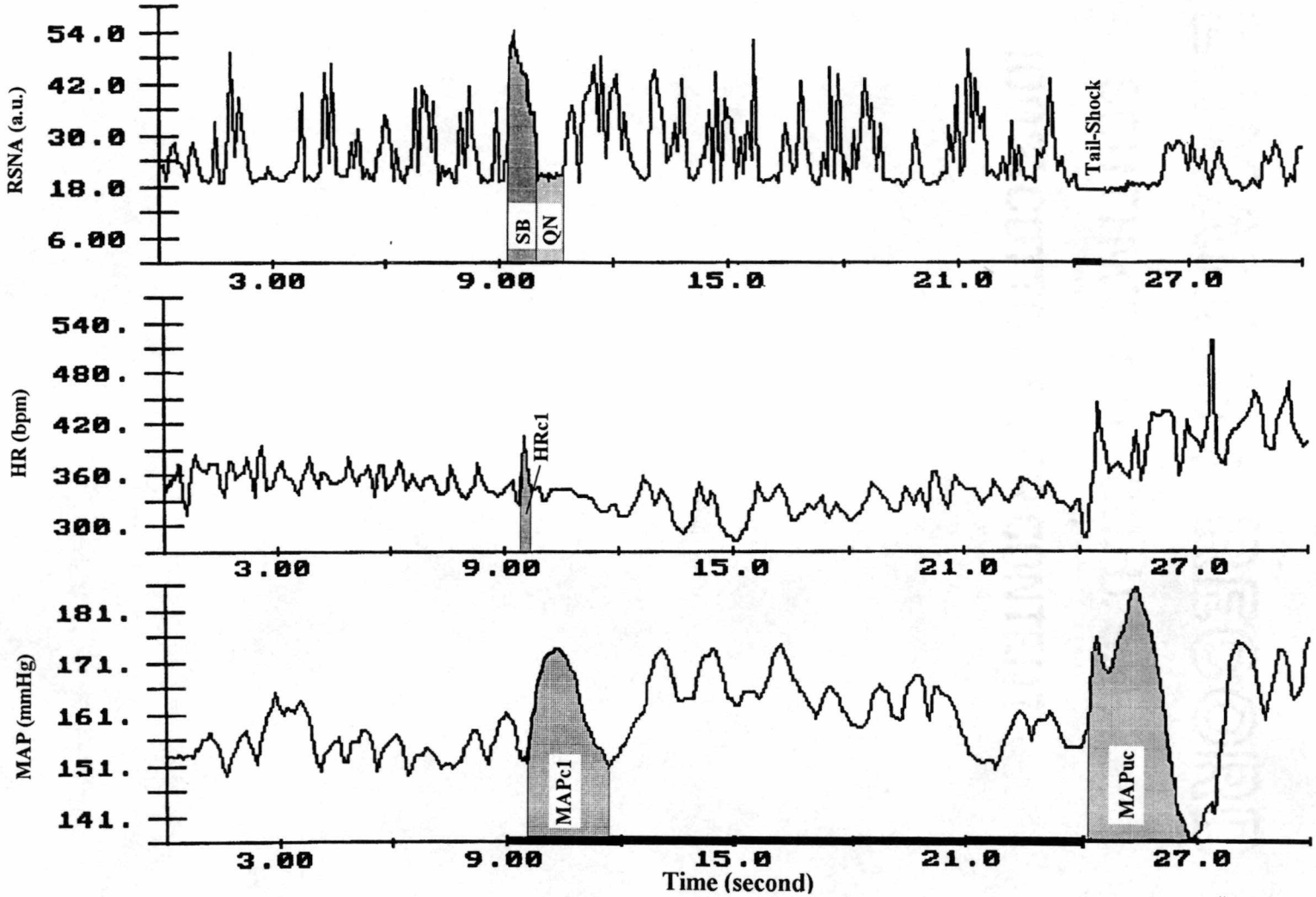

Figure 2. Pattern of RSNA, HR and MAP responses during a CS+ trial (from SHR #10, day 2, trial #9). Tone presented during 9.00-23.99 sec period (dark bar).

Table 2. Time Sequence of Conditioned Responses in RSNA and MAP (Mean±S.E.) for WKY, BHR, SHR and BHR-high during CS+ and CS- Trials

	WKY		BHR		SHR		BHR-high	
Variable	CS+	CS-	CS+	CS-	CS+	CS-	CS+	CS-
SB_{onset} (sec)	.16	.11	.16	.12	.16	.11	.16	.11
	.01	.01	.01	.01	.01	.01	.02	.01
SB_{len} (sec)	.47	.46	.56	.58	.45	.53	.57	.58
	.02	.02	.03	.02	.01	.03	.02	.02
$C1_{onset}$ (sec)	.46	.59	.36	.62	.45	.59	.38	.64
	.03	.03	.03	.03	.02	.03	.03	.04
$C1_{len}$ (sec)	1.36	1.07	1.42	1.53	1.30	1.29	1.35	1.12
	.07	.10	.07	.05	.05	.05	.05	.06

SB_{onset}: latency time of RSNA sudden burst following the tone onset;
SB_{len}: duration of RSNA sudden burst;
$C1_{onset}$: latency time of first conditioned MAP following tone onset;
$C1_{len}$: duration of C1 MAP response.

period of delay from tone onset in all groups (.16±.01 sec in WKY, .16±.01 sec in BHR, .16±.01 sec in SHR, and .16±.02 sec in BHR-high during CS+ trials; .11±.01 sec in WKY, .12±.01 sec in BHR, .11±.01 sec in SHR during CS- trials). There were no significant differences in sudden burst onset time between groups in either CS+ or CS- trials. However, the onset time was about .05 sec earlier during CS- than CS+ trials in all groups. Analysis of variance revealed a significant effect of trial type [$F(1,71)=47.31$, $p<.001$], but no significance for strain [$F(3,71)=.46$, $p=.72$]. The interaction of group x trial type was not significant [$F(3,71)=.27$, $p=.85$].

This sudden burst lasted for about a half sec (.47±.02 sec in WKY, .56±.04 sec in BHR, .45±.01 sec in SHR, and .57±.02 sec in BHR-high during CS+ trials; .46±.02 sec in WKY, .58±.02 sec in BHR, .53±.03 sec in SHR, and .58±.02 sec in BHR-high during CS- trials). Analysis of variance revealed significant effects of strain [$F(3,71)=7.22$, $p<.001$], but not trial type [$F(1,71)=.72$, $p=.40$] or the group x trial type interaction [$F(3,71)=1.81$, $p=.15$]. The sudden burst of RSNA was followed by a quiet neural traffic

period (referred to as QN in Figure 2).

At the time of the disappearance of the RSNA sudden burst, the first conditioned response (C1) in MAP (referred to as MAP_{c1} in Figure 2) began (.46±.03 sec in WKY, .36±.03 sec in BHR, .45±.02 sec in SHR, and .38±.03 sec in BHR-high during CS+ trials; .59±.03 sec in WKY, .62±.03 sec in BHR, .59±.03 sec in SHR, and .64±.04 in BHR-high during CS- trials following the tone onset). Analysis of variance revealed significant effects of trial type [$F(1,93)=107.61$, $p<.001$], and a group x trial type interaction [$F(3,93)=3.65$, $p<.05$], but there was no significant effect of strain [$F(3,93)=.73$, $p=.54$]. The onset latency of first conditioned MAP response was significantly shorter during CS+ than CS- trials in all groups ($p<.01$ in WKY, and $p<.001$ in SHR, BHR and BHR-high). During CS+ trials, the latency in SHR and WKY was longer than BHR and BHR-high (all $p<.05$). There were no significant differences between groups during CS- trials.

This C1 response in MAP lasted slightly longer than 1 sec (1.36±.07 sec in WKY, 1.42±.07 sec in BHR, 1.30±.05 sec in SHR, and 1.35±.05 sec in BHR-high during CS+ trials;

1.07±.10 sec in WKY, 1.53±.05 sec in BHR, 1.29±.05 sec in SHR, and 1.12±.06 in BHR-high during CS- trials). Analysis of variance revealed a significant effect of strain [$F(3,93)=1.23$, $p<.001$], and a strain x trial type interaction [$F(3,93)=3.21$, $p<.05$], but no effect of trial type [$F(1,93)=1.03$, $p=.31$].

There was also an abrupt C1 response in HR (referred to as HR_{c1} in Figure 2), lasting about .10 sec, during the increasing phase of the RSNA sudden burst. This abrupt HR increase was replaced by a bradycardia even before the RSNA sudden burst reached its peak. The time sequence of this HR response in C1 was not quantified because it was relatively inconsistent between trials.

Following the quiet period, a secondary (C2) conditioned response in RSNA and MAP developed gradually in 2-3 sec, while HR continued to decrease in all groups and both trial types (especially CS+ trials). These responses lasted until the termination of the tone. Part of C2, the 15.00-23.99 sec tone period, was quantified in this study. At the end of this period, an unconditioned HR (decrease)

and MAP (increase) response to tail-shock were recorded for CS+ trials. This was followed by a depressor/tachycardic response. The recording of RSNA was automatically suspended for a 3 sec period immediately before and after shock to avoid damage to amplifiers. Neural traffic recovered gradually following tail-shock. However, the RSNA following the termination of the tone was not quantified because of the interruption caused by the tail-shock.

II. INFLUENCE OF GENETIC PREDISPOSITION

RSNA, HR, and MAP responses during the different periods described above were quantified in order to examine genetic influences in WKY, BHR and SHR. The relationship between these measures is also described for the three strains.

Renal Sympathetic Nerve Activity of WKY, BHR and SHR

The RSNA responses of WKY, BHR and SHR during different periods are illustrated in Table 3 and Figures 3-6. The absolute value of RSNA is measured in artificial units (a.u.) which are converted from microvolts. Changes in RSNA

Table 3. RSNA Responses (Mean±S.E.) for WKY, BHR and SHR during CS+ and CS- Trials

	WKY (N=9)		BHR (N=9)		SHR (N=11)	
Variable	CS+	CS-	CS+	CS-	CS+	CS-
NT_{rest} (a.u.)	30±6	30±6	26±5	26±5	34±8	34±8
NT_{sb} (a.u.)	61±11	51±10	59±12	54±11	99±23	63±11
ΔNT_{sb} (%)	105±13	75±13	129±15	104±16	177±22	102±17
NT_{qn} (a.u.)	26±5	3±1	15±2	5±1	29±5	19±12
ΔNT_{qn} (%)	-13±10	-18±6	-36±6	-38±7	2±15	-29±9
NT_{tone} (a.u.)	33±6	30±5	28±6	27±6	43±9	36±7
ΔNT_{tone} (%)	14±6	1±4	5±5	1±4	26±6	6±7
$\Delta NT_{sb}/MAP_{c1}$	33±10	-3±3	24±3	-3±4	15±3	2±2

NT_{rest}: baseline RSNA;
NT_{sb}: RSNA during sudden burst;
ΔNT_{sb}: RSNA change (%) from baseline during sudden burst;
NT_{qn}: RSNA during quiet period following sudden burst;
ΔNT_{qn}: RSNA change (%) from baseline during quiet period;
NT_{tone}: RSNA during tone period (sec 15.00-23.99);
ΔNT_{tone}: RSNA change (%) from baseline during tone period;
$\Delta NT_{sb}/MAP_{c1}$: ratio of NTsb% over first conditioned MAP response;
a.u.: artificial units converted from microvolts.

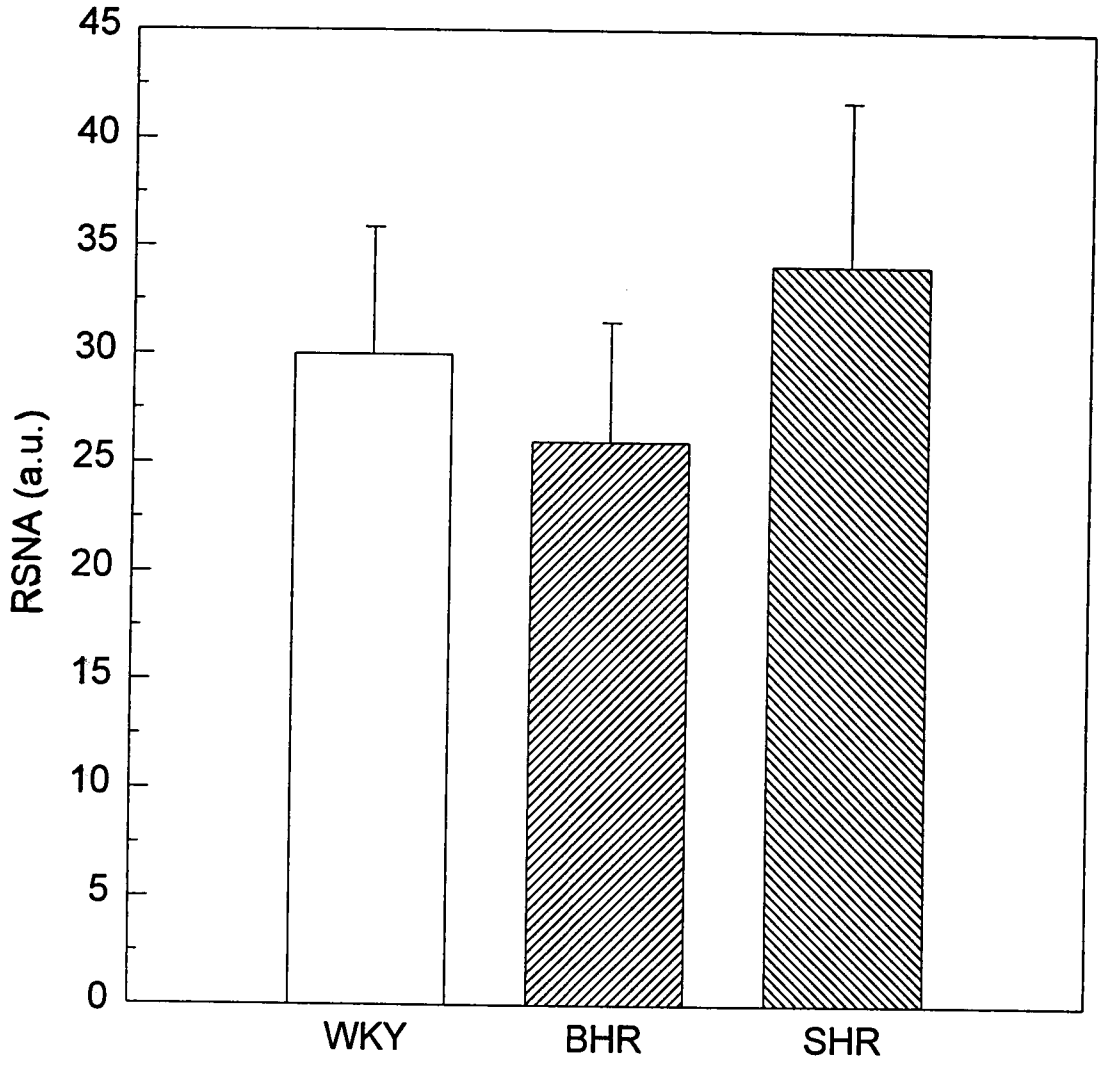

Figure 3. Baseline RSNA for WKY, BHR and SHR. Anova test: n.s. F(2,57)=.88, p=.43 between groups.

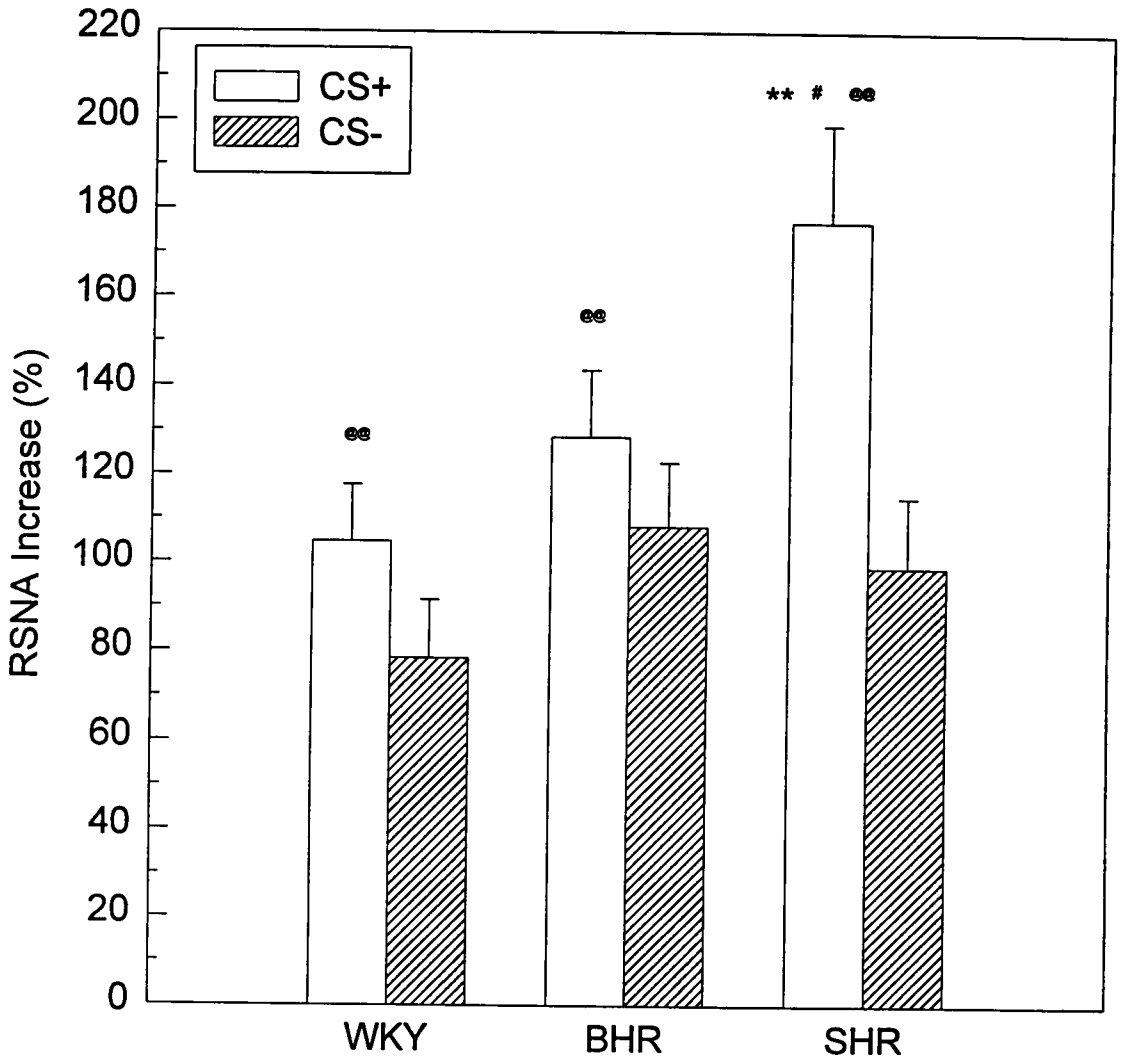

Figure 4. RSNA increases over baseline during sudden burst period for WKY, BHR and SHR during CS+ and CS- trials. Anova test: effect of strain $F(2,57)=4.15$, $p<.05$; trial type $F(1,57)=9.89$, $p<.01$.** $p<.01$ compared to WKY; * $p<.05$ compared to BHR; ●● $p<.01$ compared to CS- within groups.

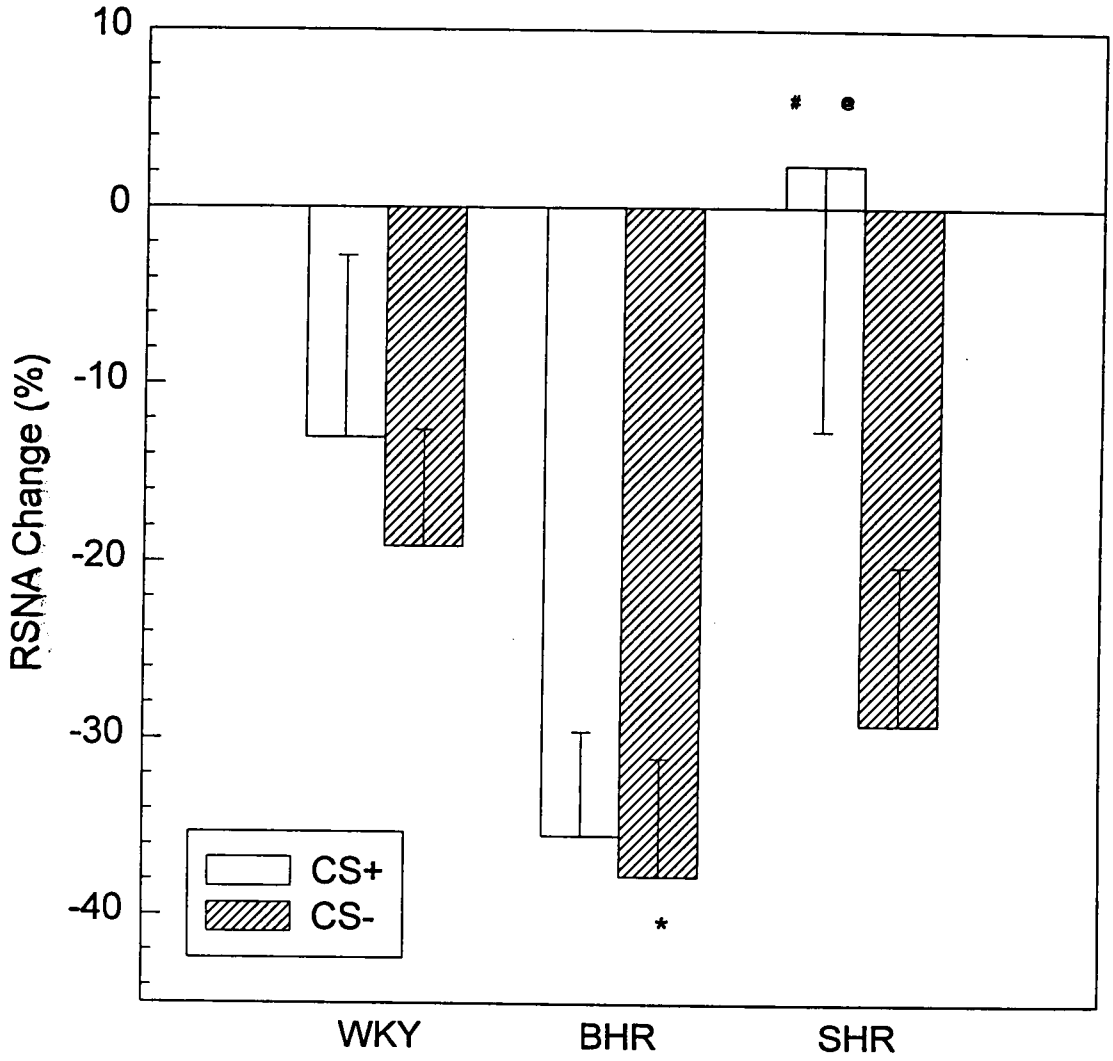

Figure 5. RSNA changes from baseline during quiet period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,56)=4.28$, $p<.05$; Anova test $p<.05$; effect of trial type: $F(1,56)=2.78$, $p=.10$. *$p<.05$ compared to WKY; # $p<.05$ compared to BHR; • $p<.05$ compared to CS- within groups.

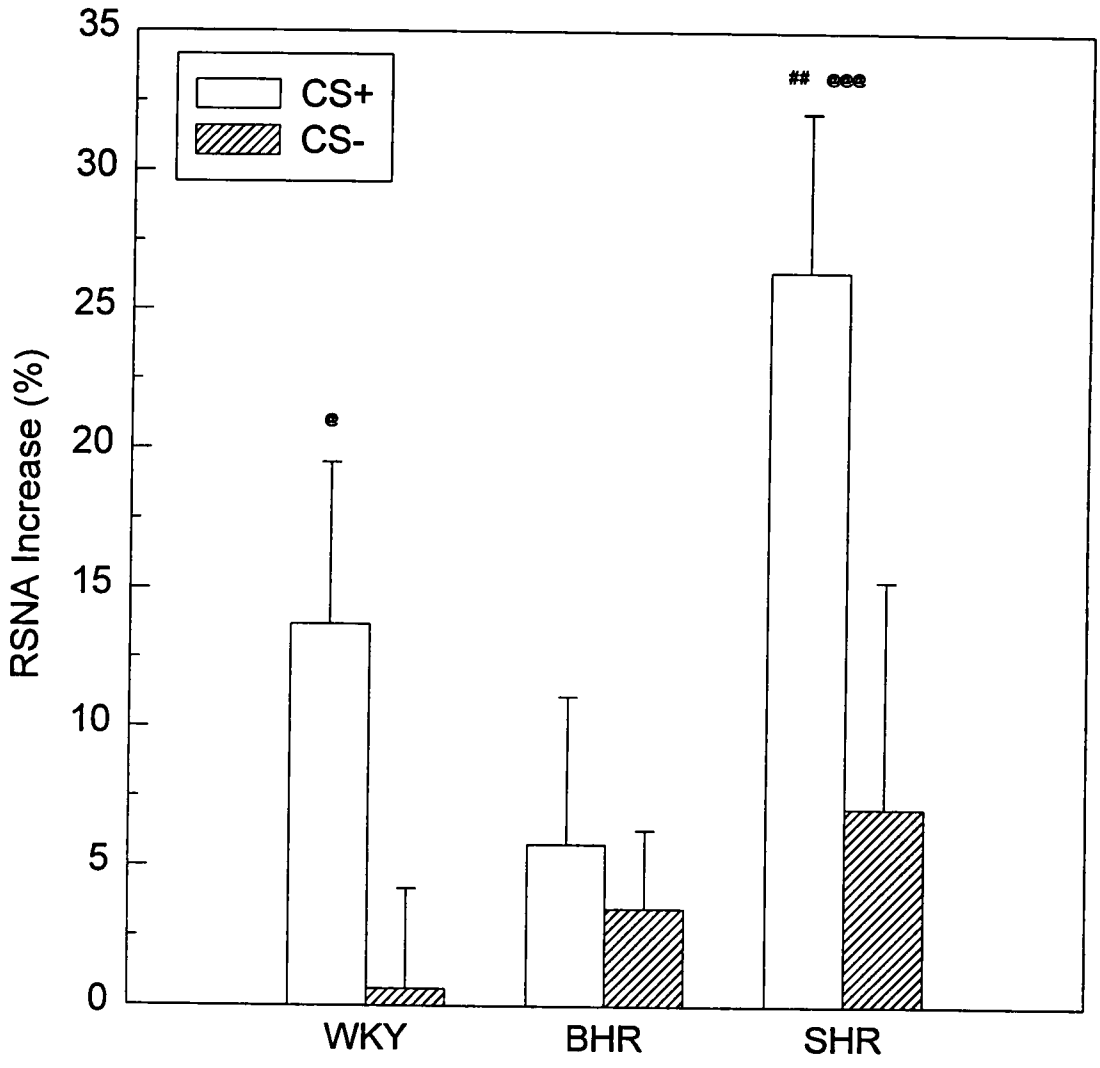

Figure 6. RSNA increases over baseline during tone period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain $F(2,57)=2.68$, $p=.08$; trial type: $F(1,57)=6.61$, $p<.05$. ## $p<.01$ compared to BHR; • $p<.05$, ••• $p<.001$ compared to CS- within groups.

for different periods are expressed as percent increase (or decrease) from baseline.

RSNA baseline showed a relatively large variance in each of the three groups. Baselines for each trial type were identical in all animals. There were no significant differences among groups [$F(2,57)=.88$, $p=.43$] in RSNA baseline (30±6 a.u. in WKY, 26±5 a.u. in BHR, and 34±8 a.u. in SHR).

The percent increase in RSNA during the sudden burst period over baseline is illustrated in Figure 4. Analysis of variance revealed a significant effect of strain [$F(2,57)=4.15$, $p<.05$], and trial type [$F(1,57)=9.89$, $p<.01$], but no interaction [$F(2,2.01)=2.01$, $p=.14$]. The increase of RSNA during CS+ trials, but not CS- trials, was significantly greater in SHR (177±22%) than in WKY (105±13%, $p<.01$ compared to SHR) or BHR (129±15%, $p<.05$ compared to SHR). All three groups had a significantly (all $p<.01$) greater increase during CS+ than CS- trials (75±13% in WKY, 104±16% in BHR, and 102±17% in SHR).

The quiet neural traffic period of RSNA (QN) following the sudden burst is illustrated in Figure 5. Analysis of variance revealed a significant effect of strain [$F(2,56)=4.28$, $p<.05$], but only a marginally significant effect of trial type [$F(1,56)=2.78$, $p=.10$], and a non significant interaction [$F(2,56)=1.57$, $p=.22$]. All groups except SHR during CS+ trials had 10-40% decreases from baseline. SHR during CS+ trials, however, were still slightly above baseline (2±15%), an effect significantly ($p<.05$) greater than SHR during CS- trials (-29±9%) and BHR during CS+ trials (-36± 6%), but not when compared to WKY during CS+ trials (-13±10%). The greatest decrease of RSNA was observed during BHR CS- trials (-38±7%) which was significantly lower than WKY during CS- trials (-18±6%).

Figure 6 shows that RSNA remained above baseline during the rest of the tone period (C2, sec 15.00-23.99) in both trial types (CS+ and CS-) for all groups. Analysis of variance revealed a marginally significant effect of strain when the two trial types were pooled together [$F(2,57)=2.68$, $p<.08$], and a significant trial type [$F(1,57)=6.61$, $p<.05$], but no significant interaction [$F(2,57)=1.66$, $p=.20$]. When

the data were analyzed separately for each trial type, the magnitude of the increase in RSNA during the tone period was significantly [$F(2,29)=4.07$, $p<.05$] different between groups during CS+ (14±6% in WKY, 5±5% in BHR, and 26±6% in SHR trials), but not CS- [$F(2,28)=.44$, $p=.65$] trials (1±4% in WKY, 1±4% in BHR, and 6±7% in SHR). The RSNA increase in SHR during CS+ trials was significantly greater than BHR during CS+ trials ($p<.01$) and SHR during CS- trials ($p<.001$). WKY had greater increases in RSNA during CS+ trials than during CS- trials ($p<.05$).

Heart Rate Responses in WKY, BHR and SHR

HR was measured as beat per minute (bpm) changes from the baseline and is presented in Table 4 and Figures 7-10. The CS+ and CS- trials shared the same baseline in each animal. As suggested in Table 4 and Figure 7, similar HR baselines were observed in all three groups (352±15 bpm in WKY, 380±12 bpm in BHR, vs. 368±9 bpm in SHR). Analysis of variance revealed no significant effects of strain [$F(2,69)=2.71$, $p=.07$].

The HR response during the first conditioned response

Table 4. HR Responses (Mean±S.E.) for WKY, BHR and SHR during CS+ and CS- Trials

	WKY (N=12)		BHR (N=12)		SHR (N=12)	
Variable	CS+	CS-	CS+	CS-	CS+	CS-
HR_{rest} (bpm)	352±15	352±15	380±12	380±12	368±9	368±9
ΔHR_{c1} (bpm)	24±5	30±9	26±9	27±5	43±5	26±6
ΔHR_{qp} (bpm)	-32±5	-28±6	-23±5	-23±5	-45±7	-25±3
ΔHR_{k-1} (bpm)	56±8	58±14	49±5	50±5	87±10	51±6
ΔHR_{tone} (bpm)	-9±5	-3±2	-21±4	-6±2	-43±7	-16±3
ΔHR_{uc} (bpm)	-32±18	n.a.	-15±6	n.a.	-23±8	n.a.

HR_{rest}: baseline heart rate;
ΔHR_{c1}: Peak conditioned HR change from baseline during C1;
ΔHR_{qp}: HR change (drop) from baseline following c1;
ΔHR_{k-1}: HR change from peak to bottom;
ΔHR_{tone}: HR change from baseline during tone period;
ΔHR_{uc}: HR change during unconditioned response period;
n.a.: not applicable (no UC for CS- trials).

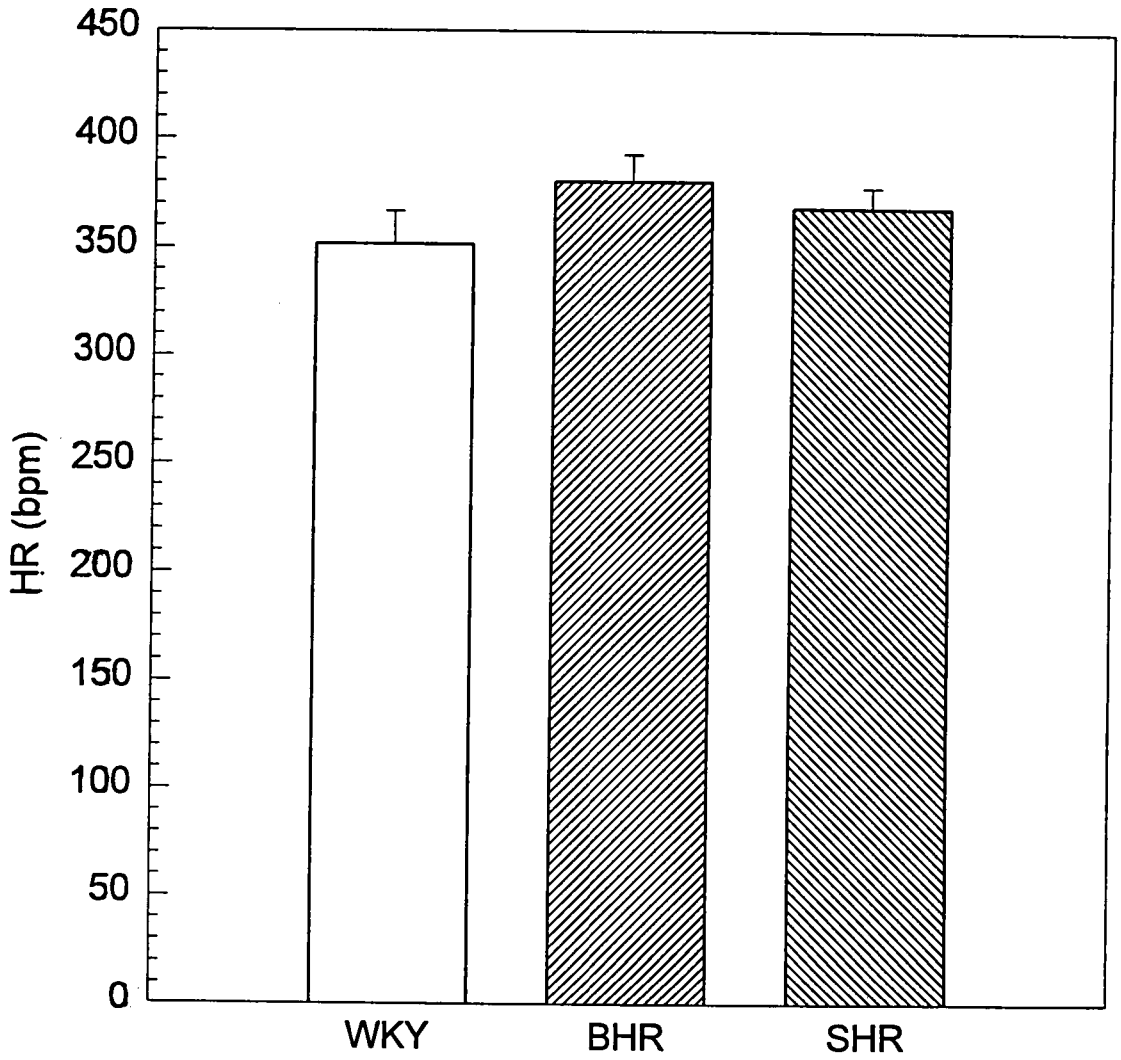

Figure 7. Baseline HR for WKY, BHR, and SHR. Effect of strain on Anova test: $F(2,69)=2.71$, $p=.07$.

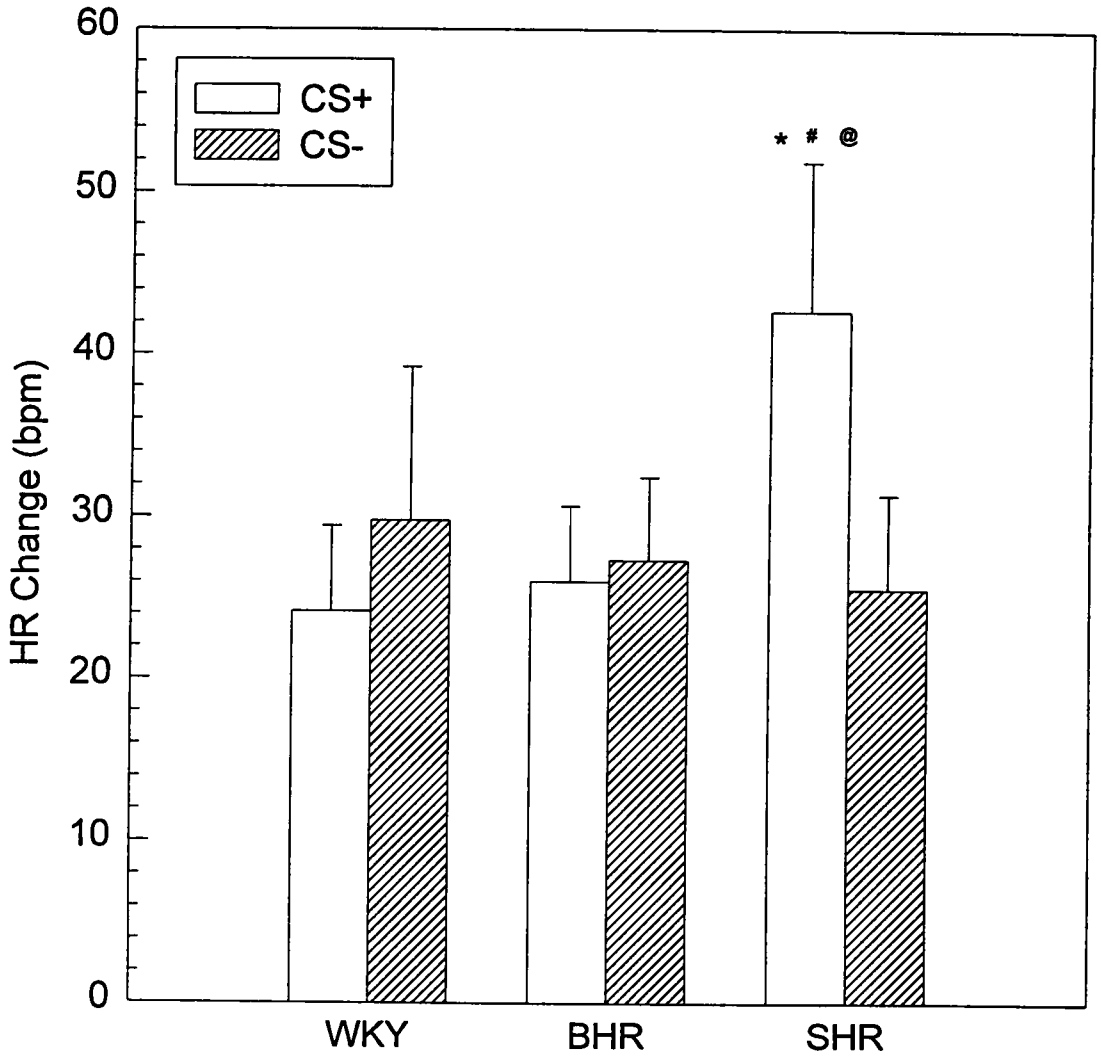

Figure 8. First conditioned HR increases over baseline for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,65)=.75$, $p=.48$; trial type: $F(1,65)=.26$, $p=.62$. *$p<.05$ compared to WKY; # $p<.05$ compared to BHR; • $p<.05$ compared to CS- within groups.

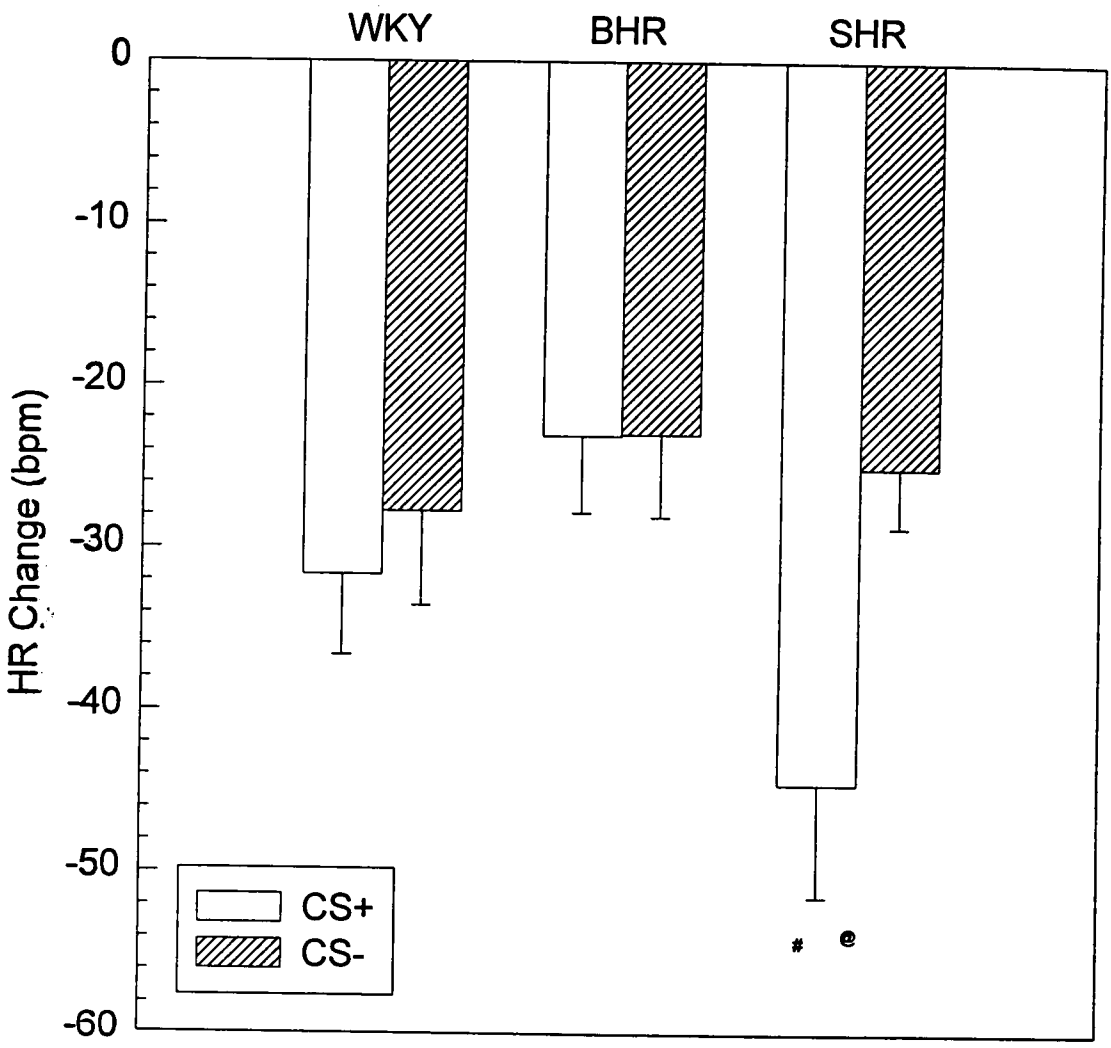

Figure 9. HR changes from baseline during the bradycardic response period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain $F(2,65)=2.5$, $p=.09$; trial type: $F(1,65)=2.82$, $p=.10$. #$p<.05$ compared to BHR; @$p<.05$ compared to CS- within groups.

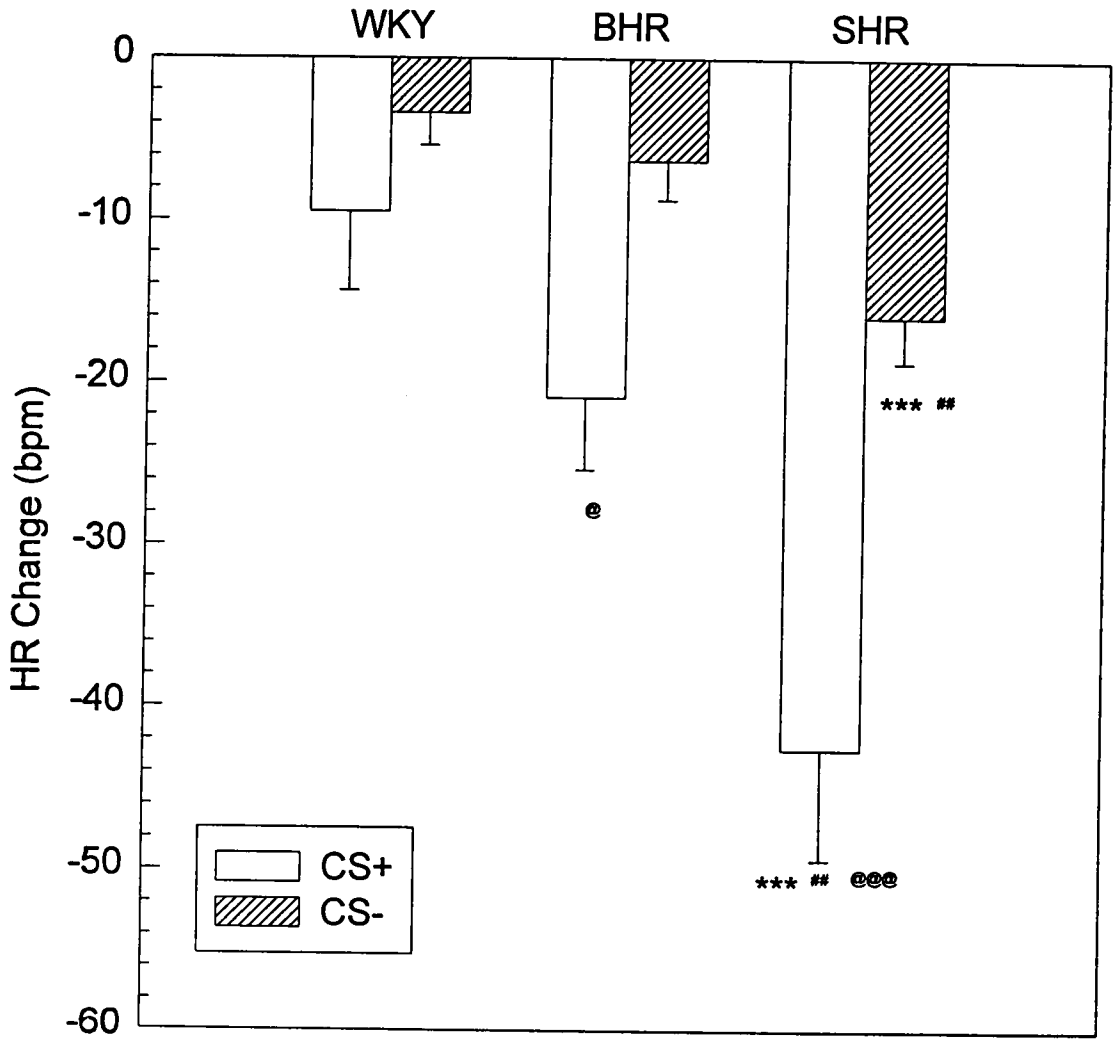

Figure 10. HR changes from baseline during tone period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,69)=15.4$, $p<.001$; trial type: $F(1,69)=20.88$, $p<.001$, *** $p<.001$ compared to WKY; ## $p<.01$ compared to BHR; • $p<.05$, and ••• $p<.001$ compared to CS- within groups.

period (C1 of Figure 2) is shown in Figure 8. There were 20-30 bpm increases in HR in each of the three groups in both trials except for SHR during CS+ trials, which showed an increase of 43±5 bpm over baseline. Analysis of variance revealed no significant effects of strain [$F(2,65)=.75$, $p=.48$], or trial type [$F(2,65)=.26$, $p=.62$], nor was there a significant strain x trial type interaction [$F(2,65)=1.52$, $p=.23$]. However, when each trial type was analyzed separately, the differences in HR during this period were marginally significant [$F(1,32)=2.39$, $p=.10$] among the three groups during CS+ trials (24±5 bpm in WKY, 26±9 bpm in BHR, vs. 43±5 bpm in SHR), but not during CS- trials (30±9 bpm in WKY, 27±5 bpm in BHR, vs. 26±6 bpm in SHR). The SHR group had a greater tachycardic response than WKY and BHR during CS+ trials, and SHR during CS- trials (all $p<.05$).

As depicted in Figure 2, and previously described (p. 97), the first conditioned HR response lasted only about .1 sec, and was followed by a sharp bradycardic response in all three groups in both trial types. Figure 9 illustrates that there was about a 20-30 bpm decrease in HR in all but SHR during CS+ trials which had an even greater decrease of

-45±7 bpm. Analysis of variance revealed a marginally significant effect of strain [$F(2,65)=2.50$, $p=.09$], and trial type [$F(1,65)=2.82$, $p=.10$], but no strain x trial type interaction [$F(2,65)=1.83$, $p=.17$]. However, when the data were tested separately for each trial type, the strain differences in HR changes were significant [$F(2,32)=3.76$, $p<.05$] for CS+ trials (-32±5 bpm in WKY, -23±5 bpm in BHR, vs. -45±7 bpm in SHR), but not [$F(2,32)=.24$, $p=.79$] for CS- trials (-28±6 bpm in WKY, -23±5 bpm in BHR trials, vs. -25±3 bpm in SHR). The HR decrease was significantly ($p<.05$) greater in SHR CS+ trials than in BHR CS+ trials and SHR CS- trials.

The HR remained below baseline in both trial types for all three groups during the rest of the tone period (sec 15.00-23.99). The differences between groups and trial types are apparent in Figure 10. Analysis of variance revealed significant effects of strain [$F(2,69)=15.40$, $p<.001$] and trial type [$F(1,69)=20.88$, $p<.001$], but only a marginal strain x trial type interaction [$F(2,69)=3.04$, $p=.05$]. During CS+ trials, SHR had a greater average HR decrease (-43±7 bpm) than WKY (-9±5 bpm, $p<.001$) and BHR (-21±4 bpm,

p<.01). SHR also had a greater average HR decrease during CS- trials (-16±3 bpm) compared to WKY (-3±2 bpm, p<.001) and BHR (-6±2 bpm, p<.01). The differences between CS+ and CS- trials were significant in SHR (p<.001) and BHR (p<.05), but not WKY.

Mean Arterial Pressure Responses in WKY, BHR and SHR

The MAP responses of WKY, BHR and SHR during different periods were measured in mmHg change from the baseline and are illustrated in Table 5 and Figures 11-15. Baselines for each trial type were identical in all animals. Analysis of variance revealed a significant effect of strain [F(2,69)= 129.99, p<.001]. Baseline MAP was significantly higher in SHR (154±3 mmHg) than in WKY (116±3 mmHg) and BHR (132±2 mmHg), both at p<.001. Also, baseline MAP was greater (p<.001) in BHR than in WKY.

The MAP response during the first conditioned response period (C1 in Figure 2) lasted about 1.3-1.5 sec in all three groups and both trial types. The data for this period are shown in Figure 12. Analysis of variance revealed significant effects of strain [F(2,68)=37.82, p<.001], and

Table 5. MAP Responses (Mean±S.E.) for WKY, BHR and SHR during CS+ and CS- Trials

	WKY (N=12)		BHR (N=12)		SHR (N=12)	
Variable	CS+	CS-	CS+	CS-	CS+	CS-
MAP_{rest} (mmHg)	116±3	116±3	132±2	132±2	154±3	154±3
ΔMAP_{c1} (mmHg)	4±1	2±1	5±1	4±1	14±2	7±2
ΔMAP_{qp} (mmHg)	-1±1	-5±1	1±1	-2±1	4±1	-6±2
ΔMAP_{k-1} (mmHg)	7±1	10±1	7±1	10±1	15±2	15±3
ΔMAP_{tone} (mmHg)	6±1	1±0	4±1	1±0	13±2	3±1
ΔMAP_{uc} (mmHg)	16±2	n.a.	15±2	n.a.	27±3	n.a.
ΔMAP_{post} (mmHg)	-10±2	n.a.	-40±1	n.a.	-2±2	n.a.

MAP_{rest}: baseline mean arterial pressure (MAP);
ΔMAP_{c1k}: first conditioned MAP response over baseline;
ΔMAP_{qp}: MAP drop from baseline after C1 response;
ΔMAP_{k-1}: MAP change from C1 peak to the following drop;
ΔMAP_{tone}: MAP change over baseline during tone period;
ΔMAP_{uc}: peak unconditioned MAP response to tail shock;
ΔMAP_{post}: depressor response following unconditioned response;
n.a.: not applicable (no UC in CS- trials).

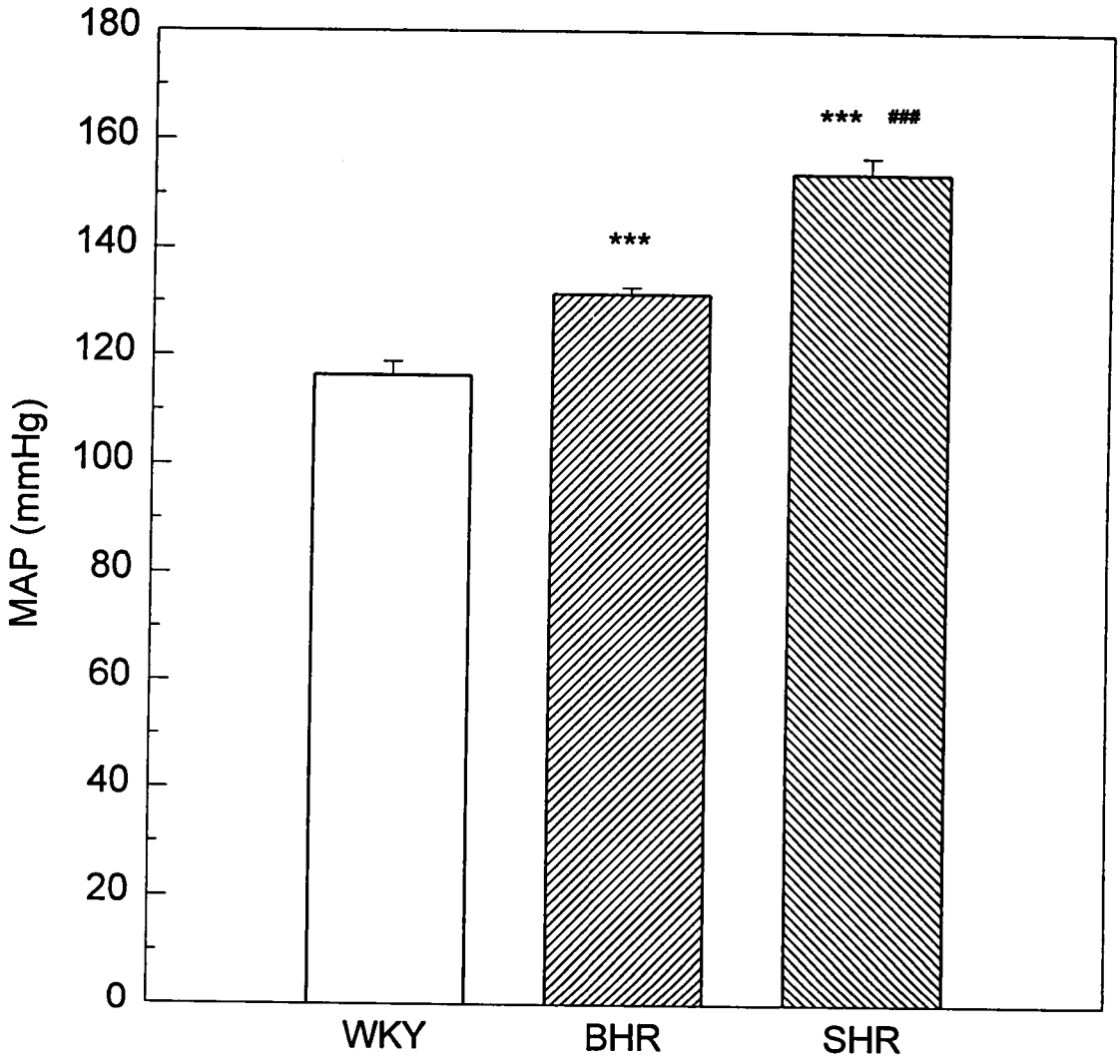

Figure 11. Baseline MAP for WKY, BHR and SHR. Effect of strain: $F(2,69)=130.0$, $p<.001$. *** $p<.001$ compared to WKY; ### $p<.001$ compared to BHR.

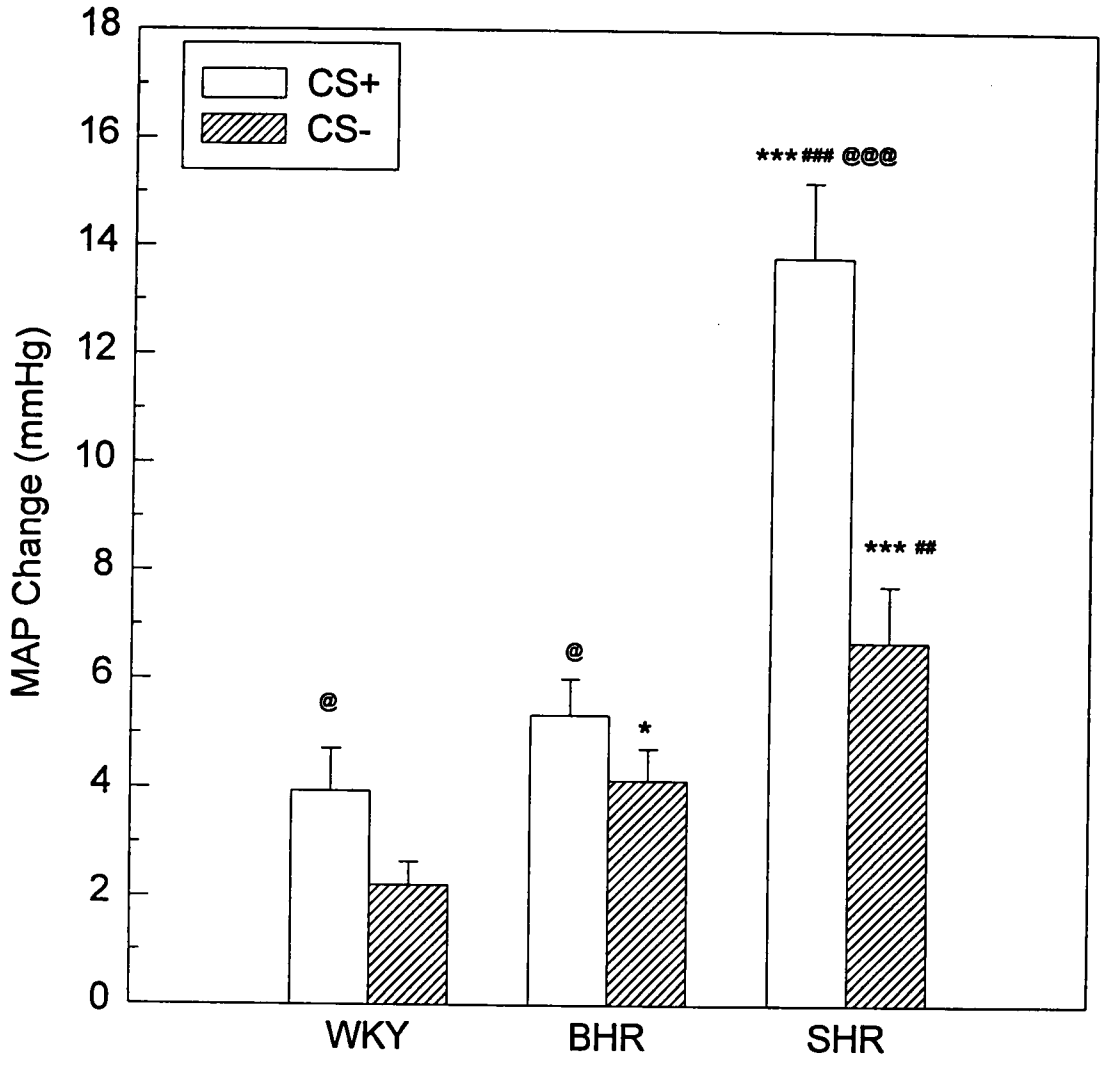

Figure 12. MAP increases over baseline during first conditioned response period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,68)=37.8$, $p<.001$; trial type: $F(1,68)=21.7$, $p<.001$. *$p<.05$, *** $p<.001$ compared to WKY; ## $p<.01$, ### $p<.00$ compared to BHR; • $p<.05$, ••• $p<.001$ compared to CS- within groups.

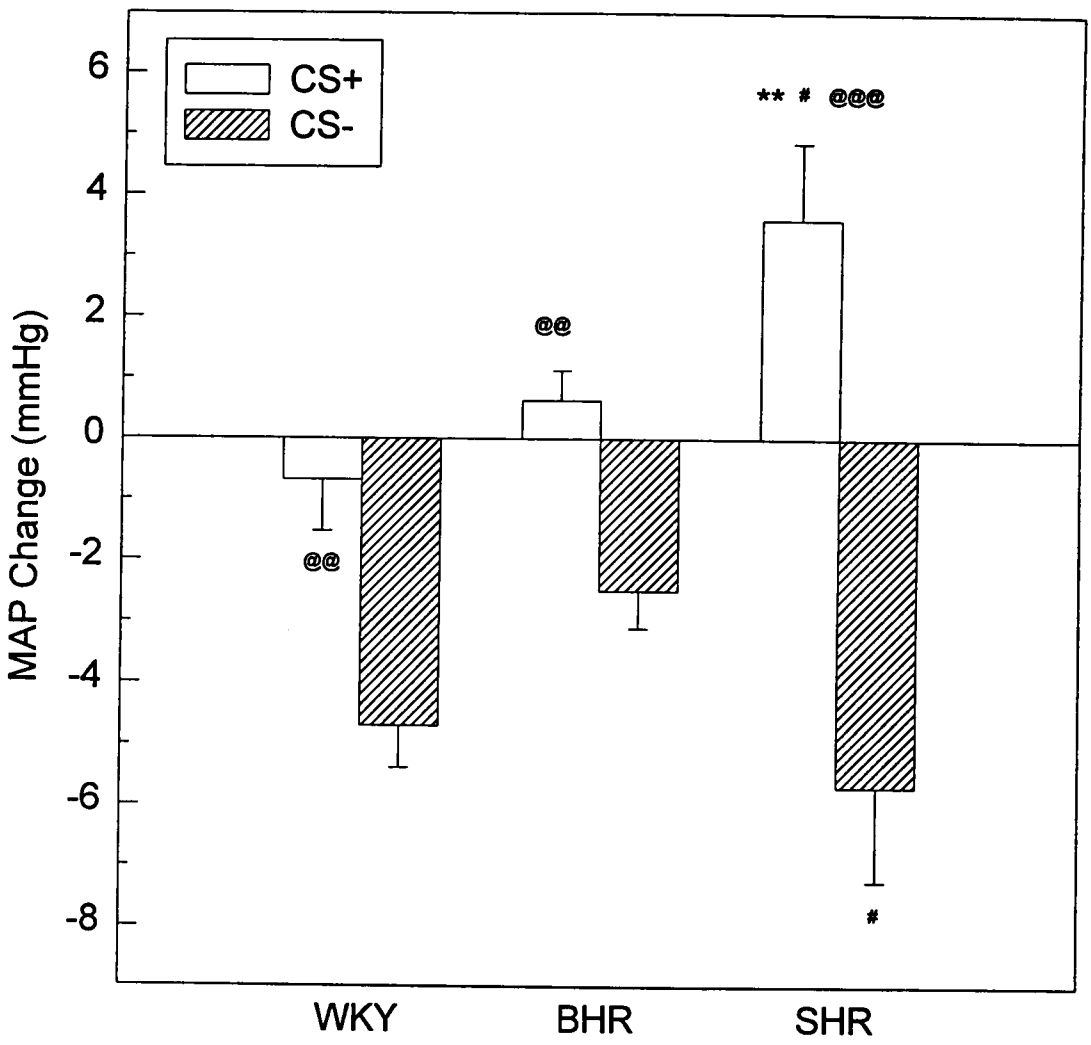

Figure 13. MAP changes from baseline during depressor response period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,69)=2.10$, $p=.13$; trial type: $F(1,69)=46.96$, $p<.001$. ** $p<.01$ compared to WKY; # $p<.05$ compared to BHR; @@ $p<.01$, @@@ $p<.001$ compared to CS- within groups.

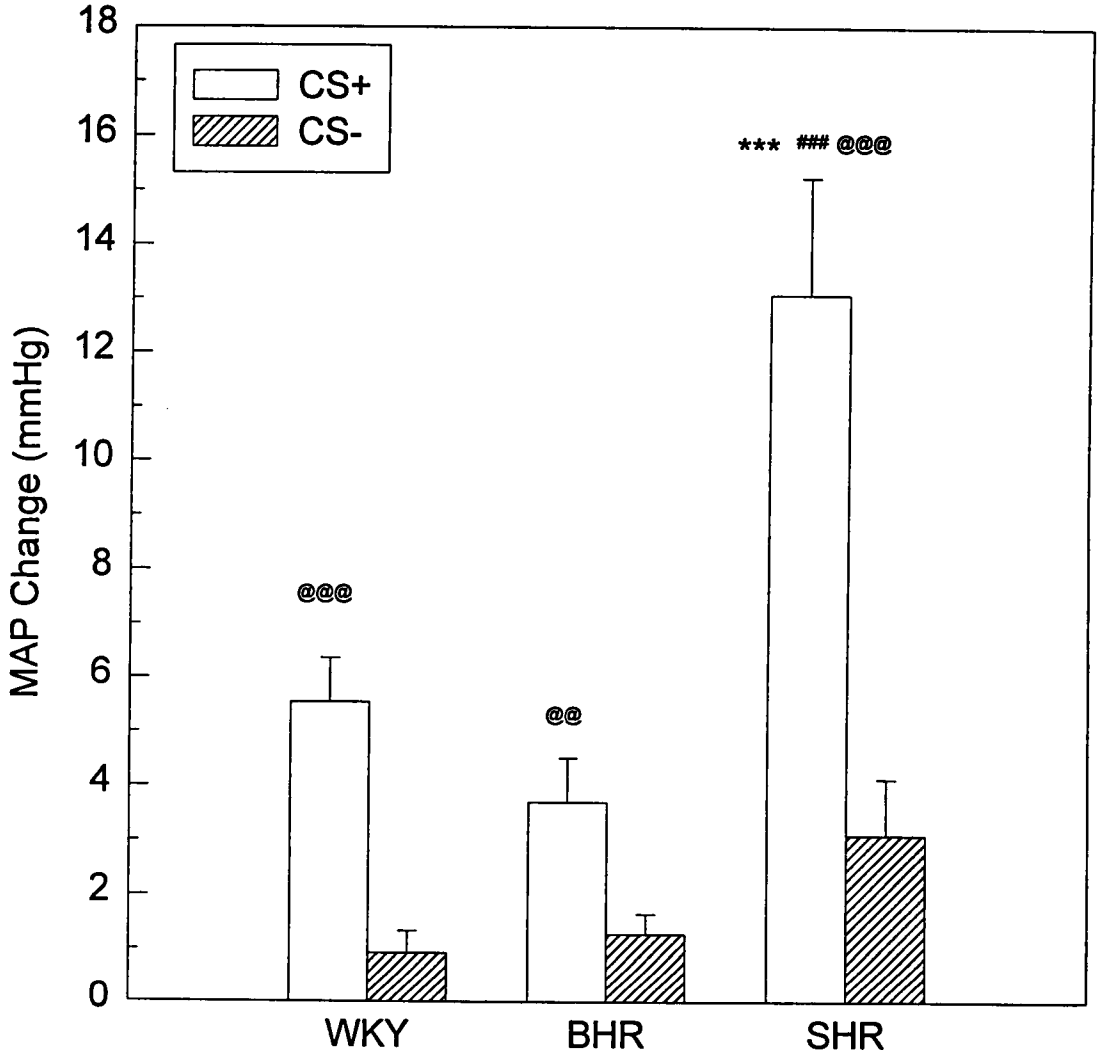

Figure 14. MAP changes from baseline during tone period for WKY, BHR and SHR during CS+ and CS- trials. Effect of strain: $F(2,68)=15.4$, $p<.001$; trial type: $F(1,68)=43.0$, $p<.001$. *** $p<.001$ compared to WKY; ### $p<.001$ compared to BHR; @@ $p<.01$, @@@ $p<.001$ compared to CS- within groups.

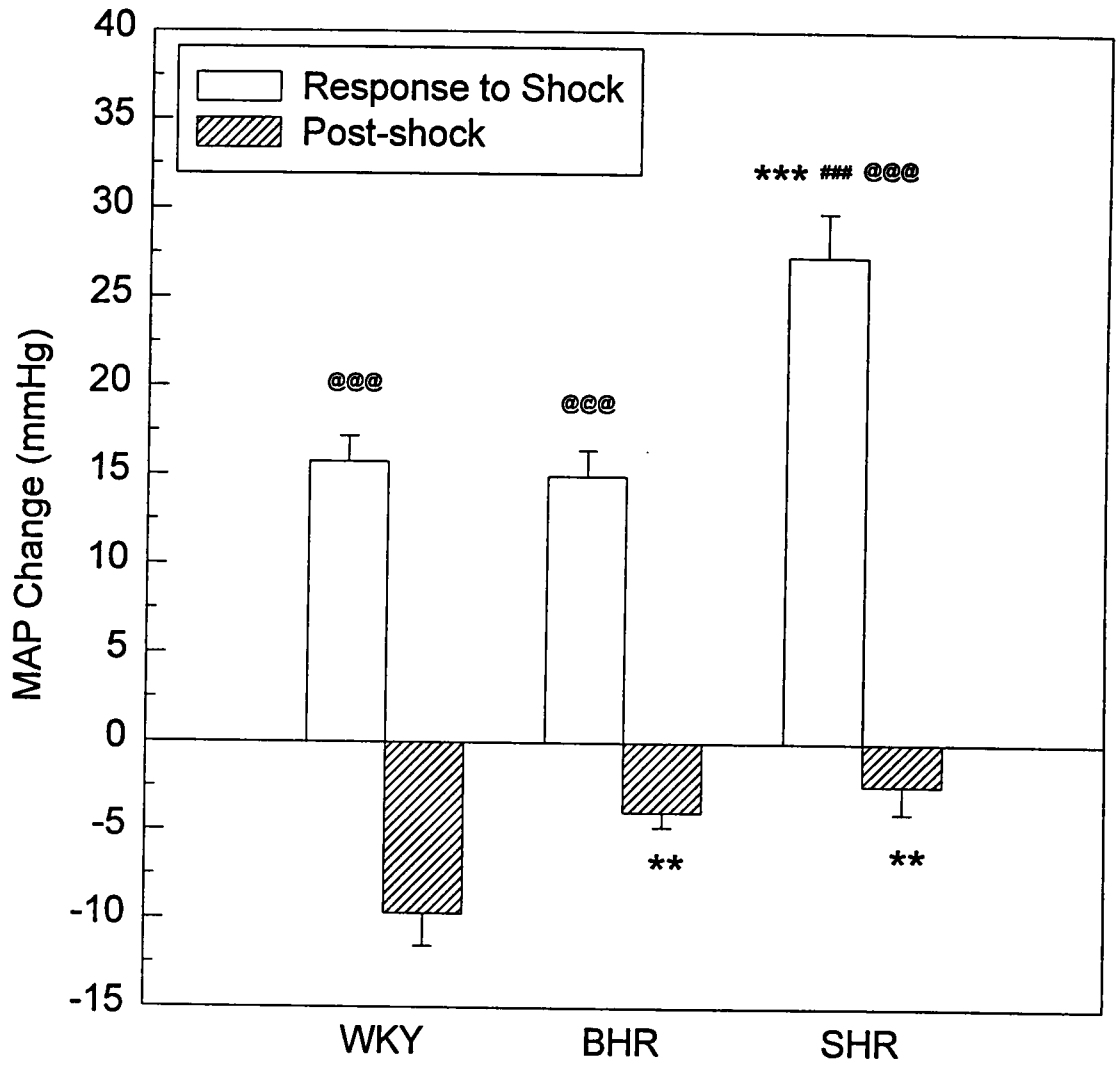

Figure 15. Peak MAP changes over baseline in response to tail-shock and during post-shock period for WKY, BHR and SHR during CS+ trials. Effect of strain $F(2,34)=14.4$, $p<.001$ for shock response; $F(2,34)=6.83$, $p<.01$ for post shock. ** $p<.01$, *** $p<.001$ compared to WKY; ### $p<.001$ compared to BHR; @@@ $p<.001$ compared to post-shock.

trial type [F(1,68)=21.67, p<.001], and a significant strain x trial type interaction [F(2,68)=7.10, p<.01]. During CS+ trials, SHR had a significantly greater pressor response during C1 (14±2 mmHg) than WKY (4±1 mmHg, p<.001) and BHR (5±1 mmHg, p<.001). Also, during CS- trials, SHR also had greater pressor responses during C1 (7±1 mmHg) than WKY (2±1 mmHg, p< .001) and BHR (4±1 mmHg, p<.01). The discrimination between CS+ and CS- trials was evident in the C1 pressor response during CS+ in all groups (p<.001 in SHR, p<.05 in both WKY and BHR).

A depressor response following the first conditioned response was observed in all animals during both trial types. These data are shown in Table 5 and illustrated in Figure 13. Analysis of variance revealed a non-significant effect of strain [F(2,69)=2.10, p=.13], a significant effect of trial type [F(1,69)=46.96, p<.001], and a significant strain x trial type interaction [F(2,69)=6.10, p<.001]. Greater depressor responses were observed during CS- (-5±1 mmHg in WKY, -2±1 mmHg in BHR, and -6±2 mmHg in SHR) than CS+ (-1±1 mmHg in WKY, 1±1 mmHg in BHR, and 4±1 mmHg in SHR) trials in all three strains (p<.01 for WKY and BHR, p<.001

for SHR). SHR had a smaller depressor response during CS+ and greater depressor response during CS- trials compared to WKY and/or BHR.

The pressor response during the rest of the tone period (sec 15.00-23.99) is shown in Figure 14. Analysis of variance revealed significant effects of strain [$F(2,68)=15.39$, $p<.001$], and trial type [$F(1,68)=43.04$, $p<.001$], and a significant strain x trial type interaction [$F(2,68)=6.94$, $p<.01$]. However, when the data were tested separately for each trial type, the strain difference was evident during CS+ [$F(2,33)=19.29$, $p<.001$], but only marginally during CS- trials [$F(2,33)=3.08$, $p=.06$]. The pressor response during CS+ trials was greater in SHR (13±2 mmHg) compared to WKY (6±1 mmHg, $p<.001$) and BHR (4±1 mmHg, $p<.001$). In contrast, essentially no pressor responses were observed for CS- trials during the tone period (1±0 mmHg in WKY, 1±0 mmHg in BHR vs. 3±1 mmHg in SHR). The discrimination between CS+ and CS- trials was again evident in the pressor response during the tone period in all groups ($p<.001$ in SHR, $p<.01$ in BHR, and $p<.001$ in WKY).

The unconditioned pressor response to tail-shock and the post-shock depressor response during CS+ trials are shown in Figure 15. Significant strain differences [$F(2,34)=14.53$, $p<.001$] were found in the unconditioned response (16±2 mmHg in WKY, 15±2 mmHg in BHR vs. 27±3 mmHg in SHR). There was also a significant strain difference [$F(2,34)=6.83$, $p<.01$] in the post-shock depressor response (-10±2 mmHg in WKY, -4±1 mmHg in BHR vs. -2±2 mmHg in SHR). A greater unconditioned pressor response to tail-shock was found in SHR than WKY and BHR (both $p<.001$), but not between WKY and BHR. The post-shock depressor response was greater in WKY compared to BHR and SHR, but not between BHR and SHR. The differences between the unconditioned response and the post-shock depressor response were significant in all groups (all $p<.001$).

Relationship among RSNA, HR and MAP Responses

Scatterplots of RSNA, HR and MAP baseline, as well as changes during the first conditioned response and the remaining tone period (sec 15.00-23.99) during CS+ trials are plotted in Figures 16-24 to show their distribution and relationship. Correlation analyses between every two

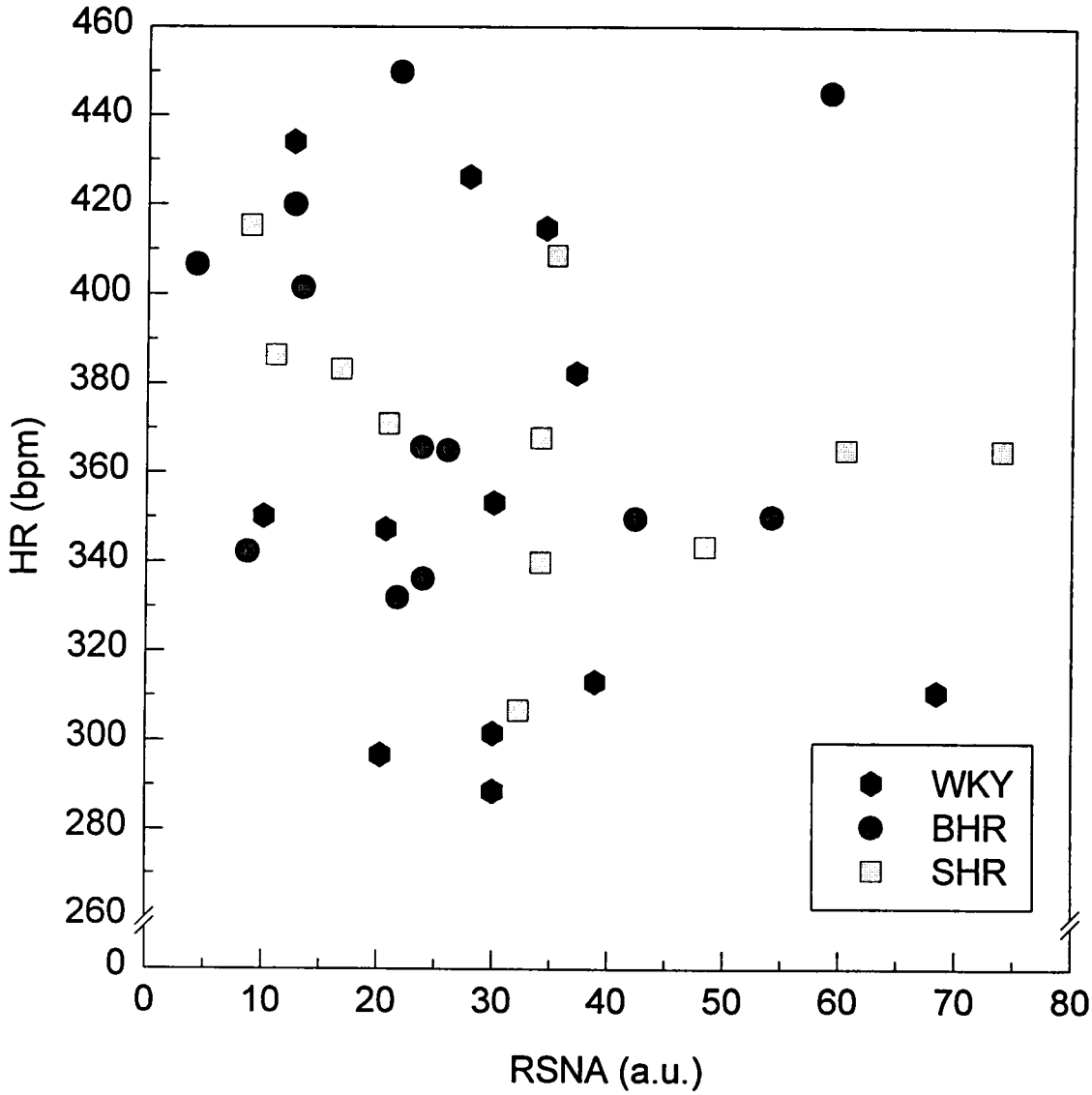

Figure 16. Scatterplot of baseline RSNA vs. HR for WKY, BHR and SHR. Correlation: r=-.0990, p=n.s.

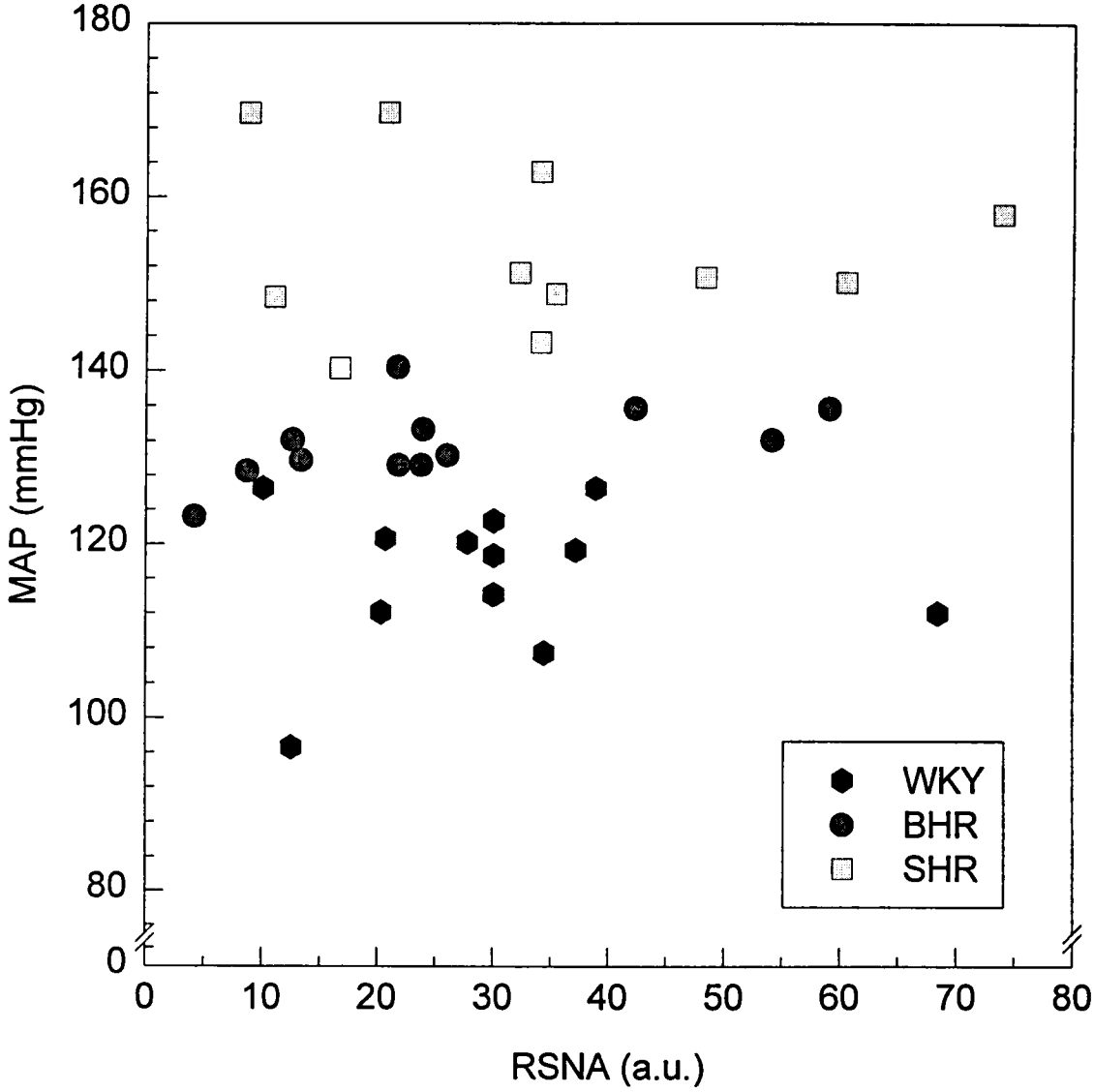

Figure 17. Scatterplot of baseline RSNA vs. MAP for WKY, BHR and SHR. Correlation: r=.1115, p=n.s.

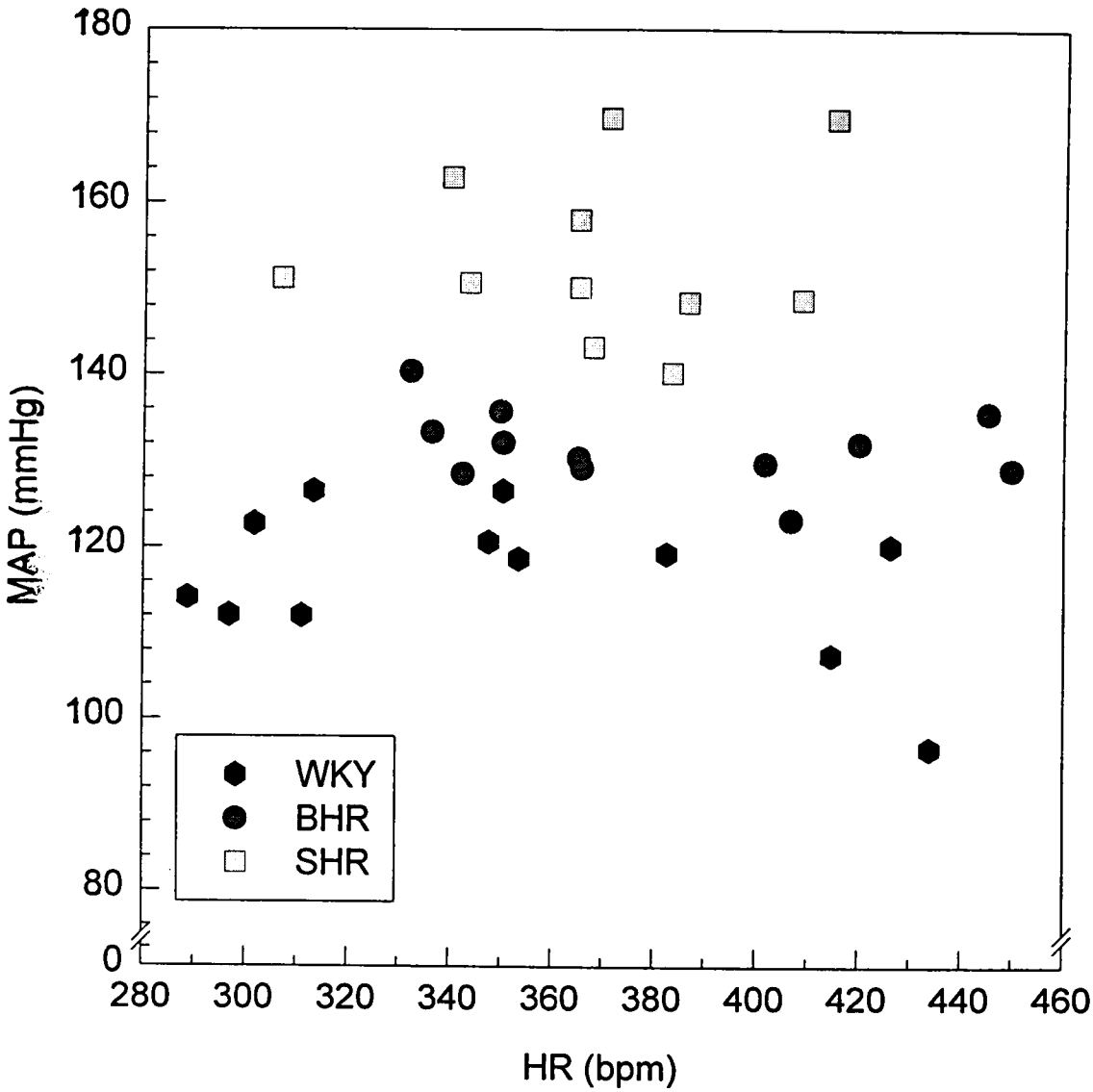

Figure 18. Scatterplot of baseline HR vs. MAP for WKY, BHR and SHR. Correlation: r=.0580, p=n.s.

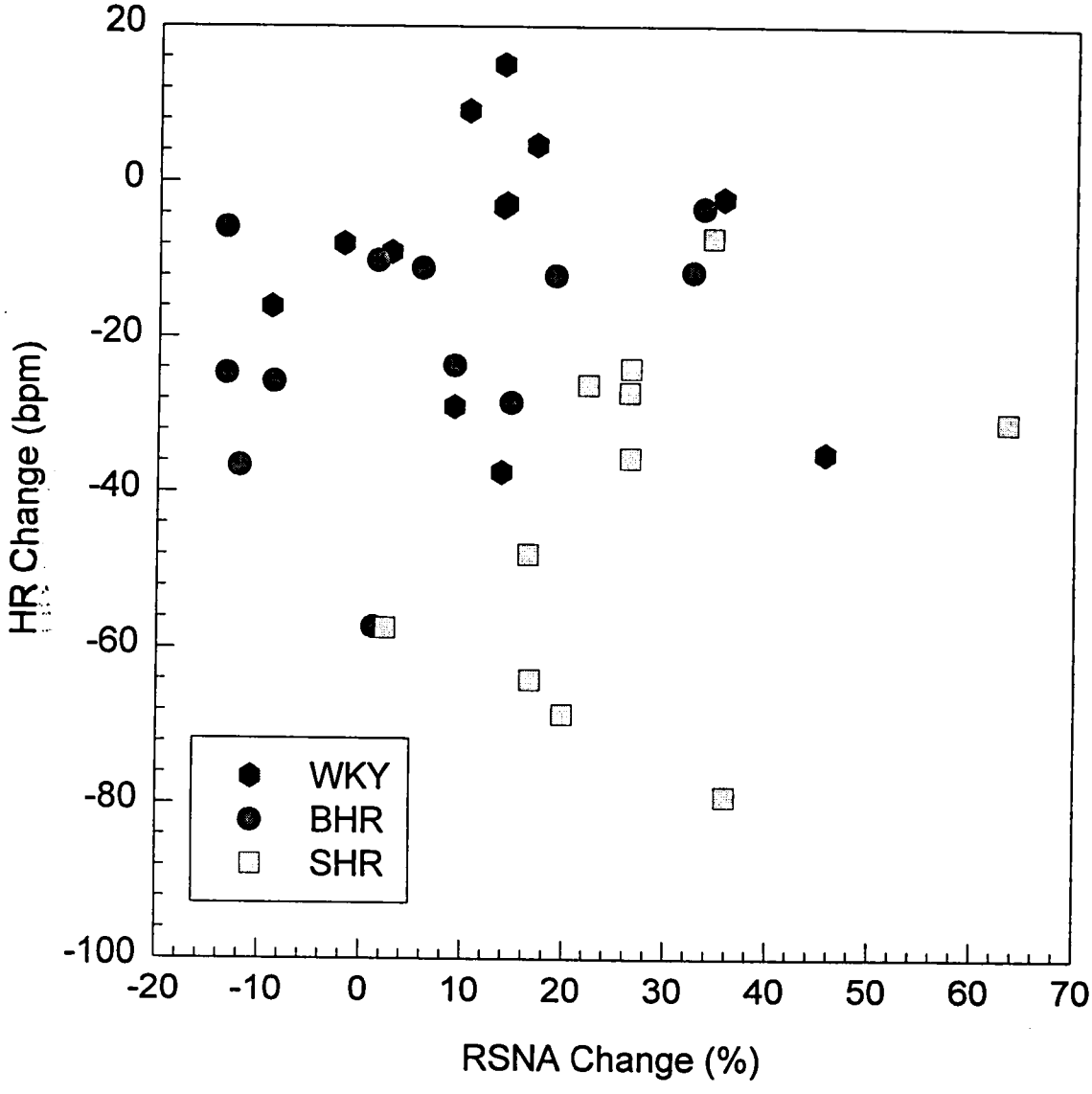

Figure 19. Scatterplot of RSNA vs. HR changes from baseline during tone period for WKY, BHR and SHR during CS+ trials. Correlation: r=-.0866, p=n.s.

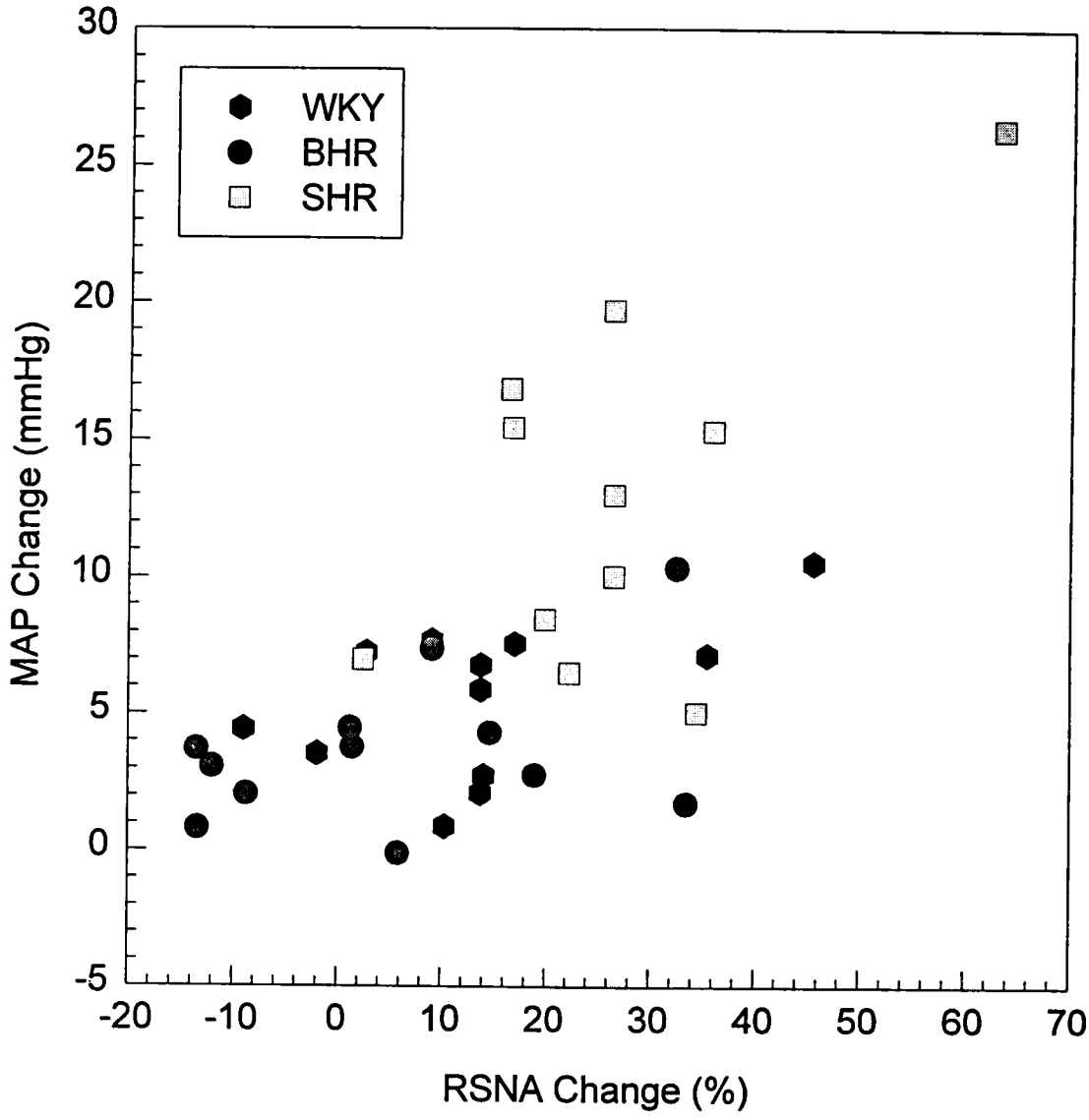

Figure 20. Scatterplot of RSNA vs. MAP changes from baseline during tone period for WKY, BHR and SHR during CS+ trials. Correlation: r=.6424, p<.001.

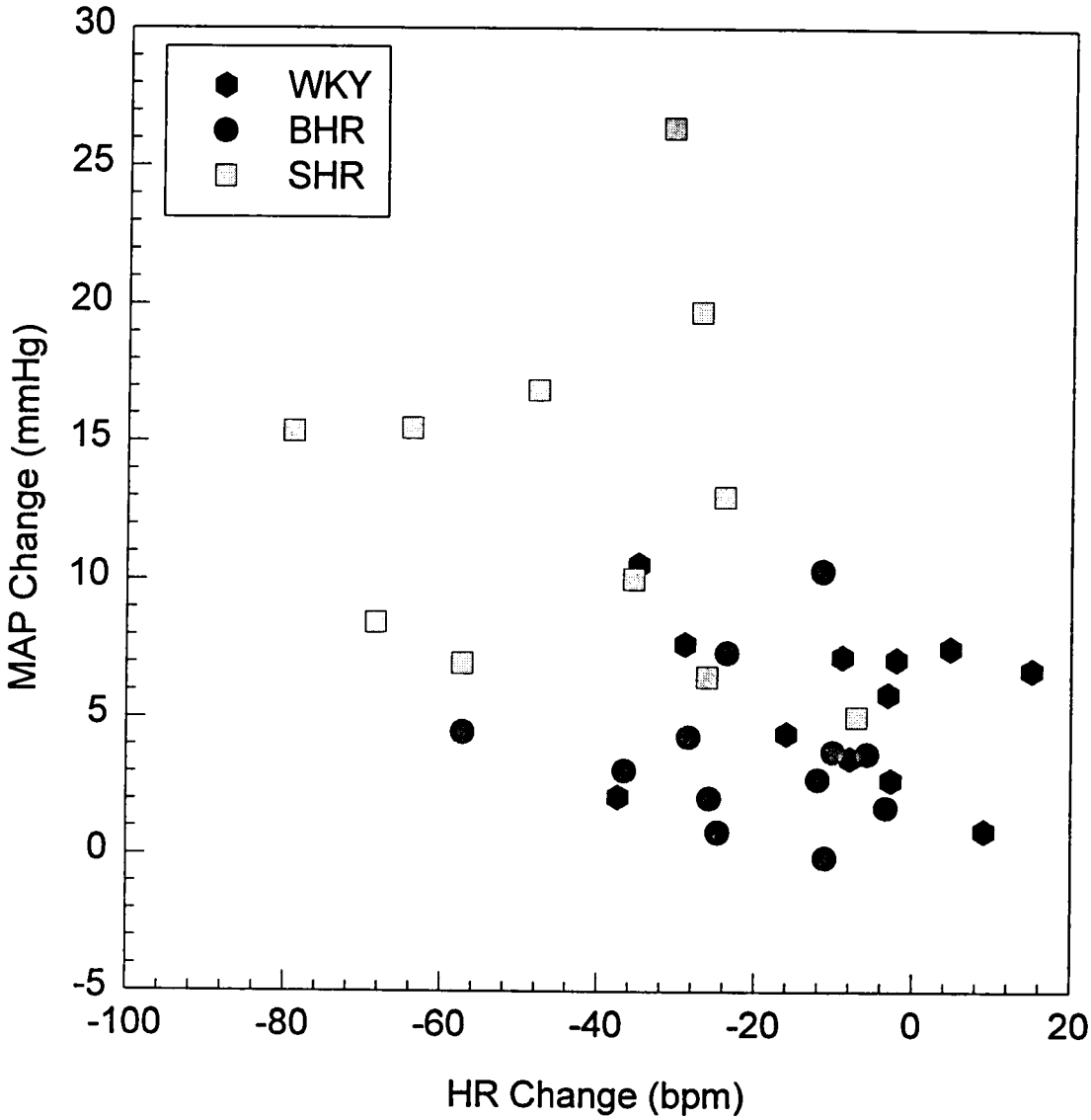

Figure 21. Scatterplot of HR vs. MAP changes from baseline during tone period for WKY, BHR and SHR during CS+ trials. Correlation: r=-.3864, p<.05.

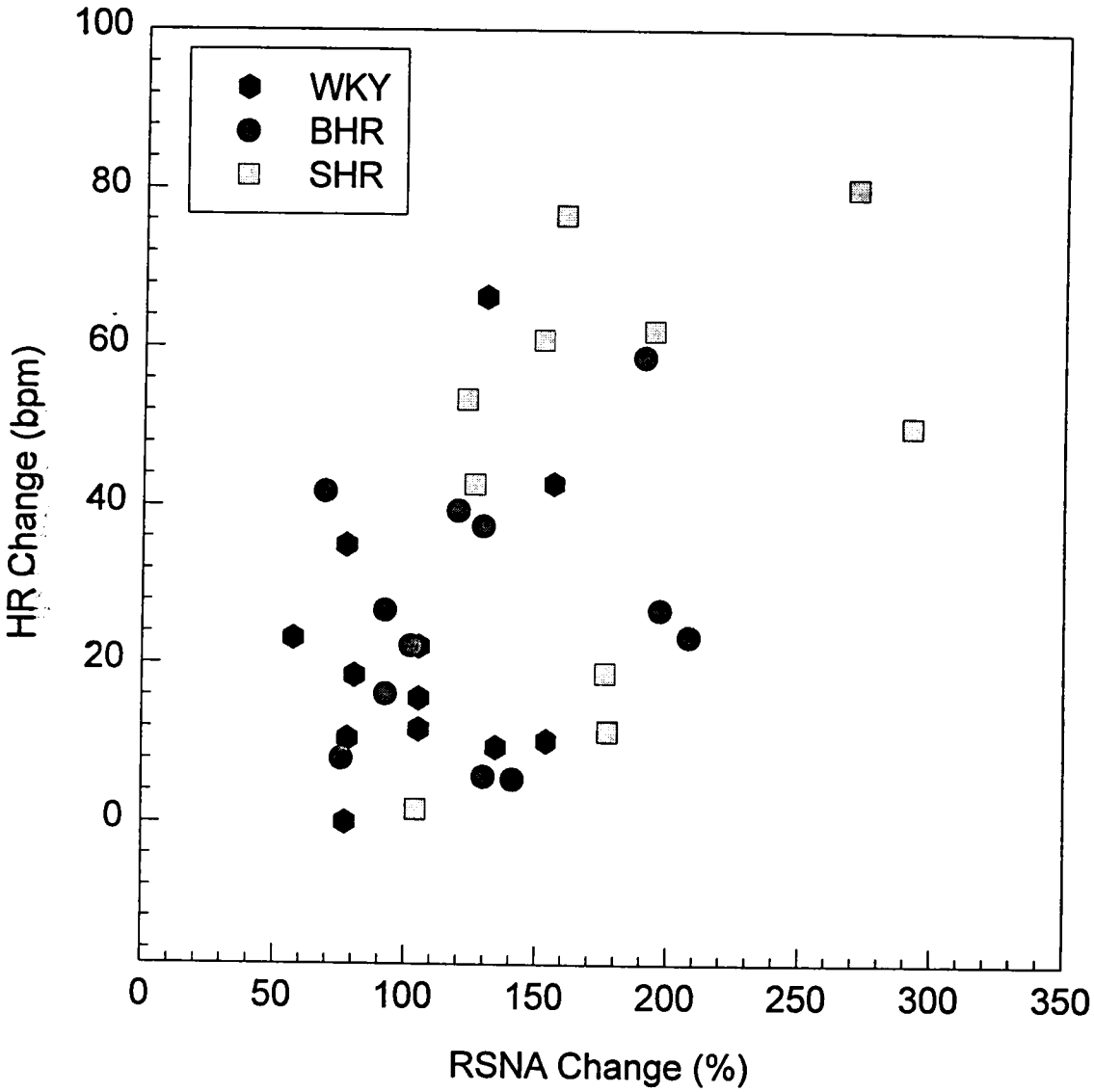

Figure 22. Scatterplot of RSNA changes during sudden burst vs. first conditioned HR changes from baseline for WKY, BHR and SHR duriung CS+ trials. Correlation: r=.4209, p<.05.

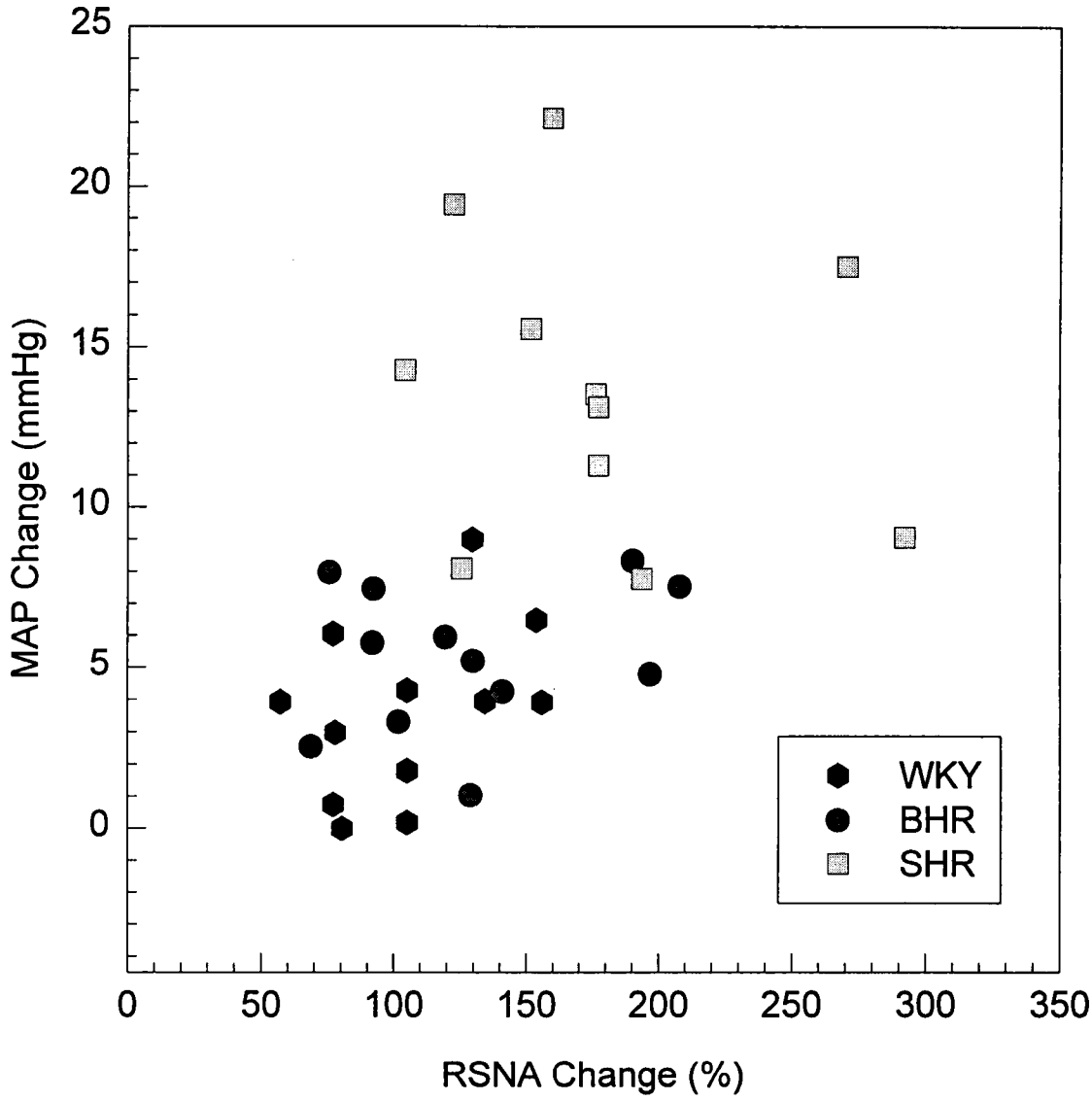

Figure 23. Scaterplot of RSNA changes during sudden burst vs. first conditioned MAP changes from baseline for WKY, BHR and SHR during CS+ trials. Correlation: r=.4061, p<.05.

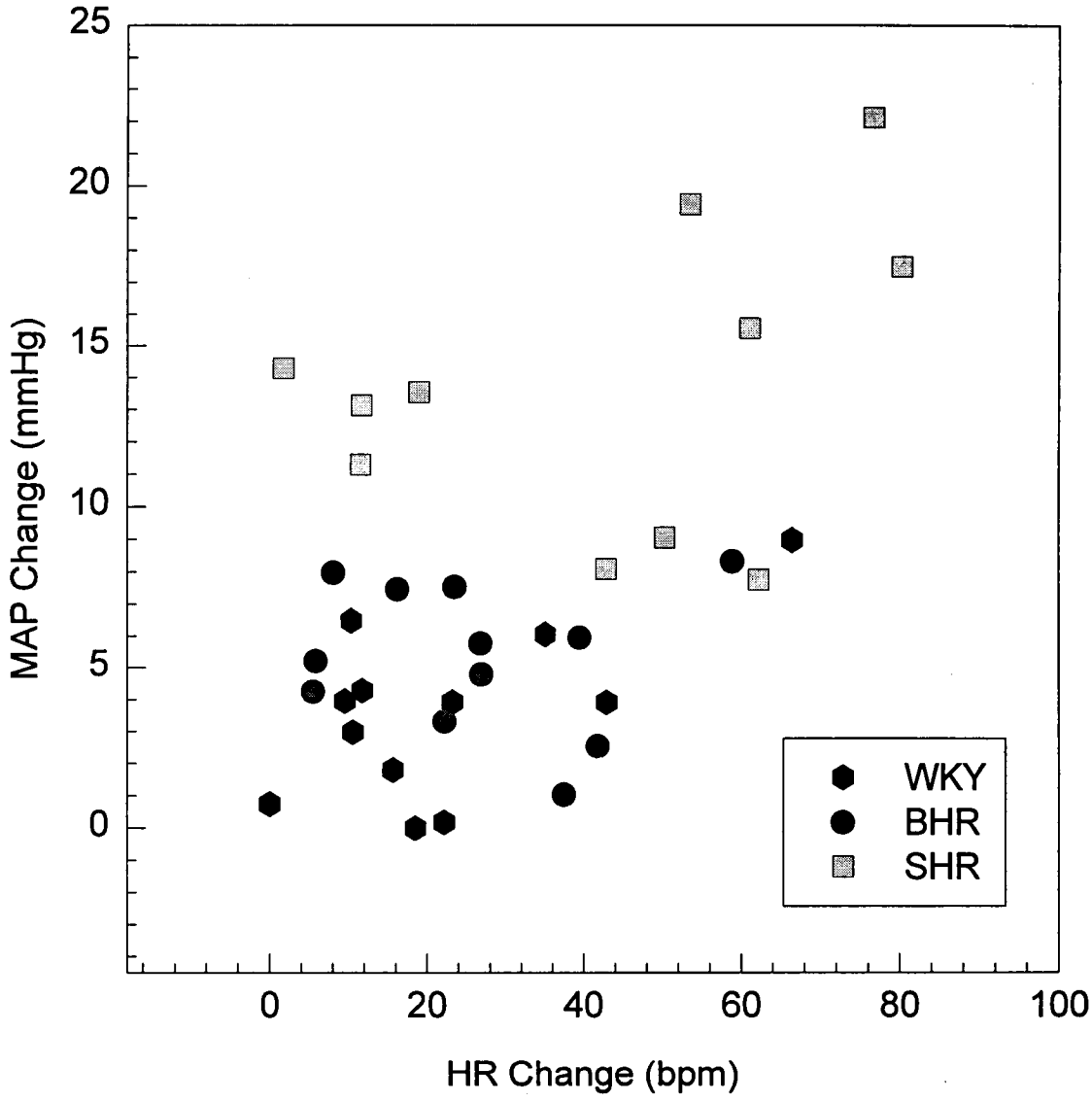

Figure 24. Scatterplot of HR vs. MAP changes from baseline during first conditioned response period for WKY, BHR and SHR during CS+ trials. Correlation: r=.4689, p<.01.

measurements during CS+ trials were carried out in a pooled three group sample. Data from CS- trials were excluded because the CS- trial is a negative control for the CS+ trial so changes were minimal.

The scatterplots for baseline RSNA, HR and MAP are illustrated in Figure 16, 17 and 18. The scatterplot for baseline RSNA and baseline HR is shown in Figure 16. The correlation (r=-.099) between them was not significant. The scatterplot for baseline RSNA and MAP is plotted in Figure 17. While the distribution of baseline MAP for the three groups was separated, the distribution of baseline RSNA was evenly scattered in all three groups. There was no significant correlation (r=.115) between baseline RSNA and MAP. Figure 18 illustrates the scatterplot for baseline HR against baseline MAP. Again, the scatterplot for baseline MAP, but not baseline HR, was separated among the three groups. The correlation (r=.0580) between baseline HR and MAP was not significant.

The scatterplots for RSNA, HR and MAP during the tone period (sec 15.00-23.99) are illustrated in Figures 19-21.

As illustrated in Figure 19, there was no significant correlation (r=-.0866) between the RSNA change and HR change during the tone period, although the scatterplot for both measurements was separated among the three groups. On the other hand, there was a significant ($p<.001$) positive correlation (r=.6424) between the percent change of RSNA from baseline and change of MAP (mmHg) during the tone period (Figure 20). Greater RSNA and MAP increases were found in SHR compared to WKY and BHR. Figure 21 shows the scatterplot of HR and MAP changes from baseline during the tone period. SHR scattered toward greater MAP increases and HR decreases while the WKY and BHR concentrated relatively in region of small MAP and HR responses. There was a significant ($p<.05$) negative correlation (r=-.3864) between the HR and MAP responses during the tone period.

The scatterplots for the first conditioned responses in HR, MAP, and RSNA increase over baseline during the sudden burst are illustrated in Figures 22-24. As shown in Figure 22, the scatterplot for RSNA against the HR response was similar to that for RSNA vs. MAP. Again, a significant ($p<.05$) positive correlation (r=.4209) was evident. As shown

in Figure 23, SHR had greater increases in sudden burst RSNA and MAP while WKY were distributed in the region with small RSNA and MAP changes. BHR were scattered between the two and partly overlapped with them. There was a significant ($p<.05$) positive relationship between RSNA and MAP during this period ($r=.4061$). Figure 24 illustrates the relationship between the first conditioned HR and MAP responses. SHR were distributed mainly in the region of greater MAP and HR increases while the WKY were in the region with small MAP and HR increases. BHR were distributed closer to WKY with some overlap with both WKY and SHR. A positive correlation ($r=.4689$) is evident between these two measurements ($p<.01$).

II. EFFECT OF HIGH DIETARY SODIUM INTAKE

Results from 12 BHR on normal salt diet (referred as BHR or BHR group) and another 12 BHR on high salt diet (referred as BHR-high or BHR-high group) are illustrated here. The HR and MAP data were collected from all 24 animals. However, RSNA was only recorded from 12 BHR and 8 BHR-high rats because of technical difficulties.

Renal Sympathetic Nerve Activity of BHR and BHR-high

The RSNA results for BHR and BHR-high groups are described in Table 6 and the percent changes from baseline during different periods are illustrated in Figures 25-28. RSNA is measured in artificial units converted from microvolts.

As shown in Figure 25, the baseline RSNA was slightly, but not significantly, smaller in BHR-high (22±4 a.u.) than in control BHR (26±5 a.u.). The percent change of sudden burst RSNA over baseline is illustrated in Figure 26. This RSNA change was greater ($p<.05$) in BHR-high group (197±19%) than in BHR (129±15%) during CS+ trials, but no significant differences between the groups were observed during CS- trials (104±16% in BHR, 87±14% in BHR-high). Increases were greater during CS+ trials compared with CS- trials in both groups ($p<.05$ in BHR, $p<.01$ in BHR-high group). The RSNA change during the quiet neural traffic period is depicted in Figure 27. Both BHR and BHR-high animals had about a 30-40% decrease in RSNA from their baseline level. However, there were no significant differences in the magnitude of the decrease between groups or trial types. Changes in RSNA in

Table 6. RSNA Responses (Mean±S.E.) for BHR and BHR-high during CS+ and CS- Trials

Variable	BHR (N=11)		BHR-high (N=8)	
	CS+	CS-	CS+	CS-
NT_{rest} (a.u.)	26±5	26±5	22±4	22±4
NT_{sb} (a.u.)	59±12	54±11	61±13	43±11
ΔNT_{sb} (%)	129±15	104±16	197±19	87±14
NT_{qn} (a.u.)	15±2	5±1	18±8	14±2
ΔNT_{qn} (%)	-36±6	-38±7	-30±17	-25±8
NT_{tone} (a.u.)	28±6	27±6	25±5	23±5
ΔNT_{tone} (%)	5±5	1±4	17±5	6±1
$\Delta NT_{sb}/MAP_{c1}$	24±3	-3±4	30±5	-2±6

NT_{rest}: baseline RSNA;
NT_{sb}: RSNA during sudden burst;
ΔNT_{sb}: RSNA change (%) from baseline during sudden burst;
NT_{qn}: RSNA during quiet period following sudden burst;
ΔNT_{qn}: RSNA change (%) from baseline during quiet period;
NT_{tone}: RSNA during tone period;
ΔNT_{tone}: RSNA change (%) from baseline during tone period;
$\Delta NT_{sb}/MAP_{c1}$: ratio of NT_{sb}% over first conditioned MAP response;
a.u.: artificial units converted from microvolt.

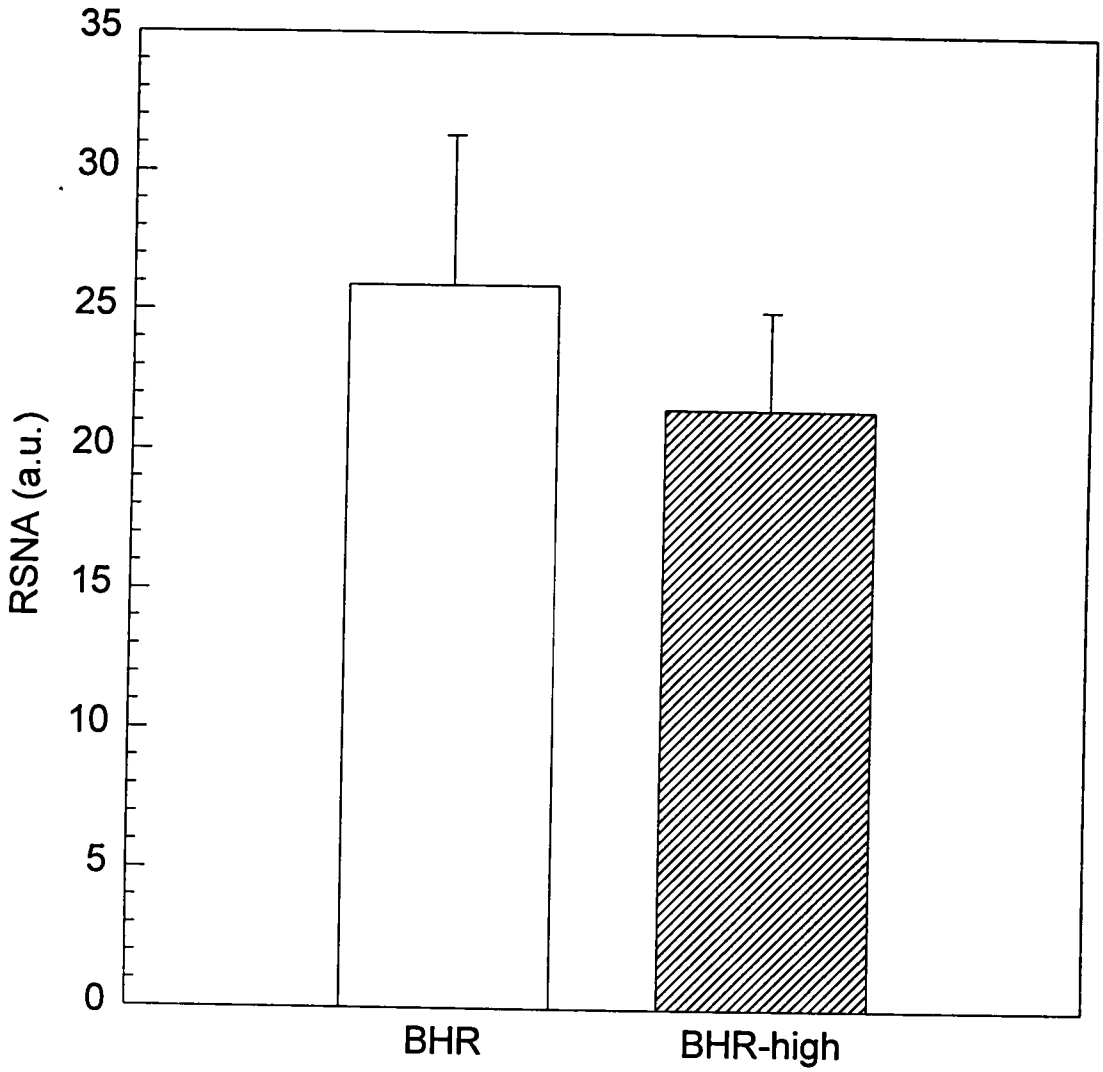

Figure 25. Baseline RSNA for BHR on normal (BHR) vs. high (BHR-high) salt diet. T-test: n.s.

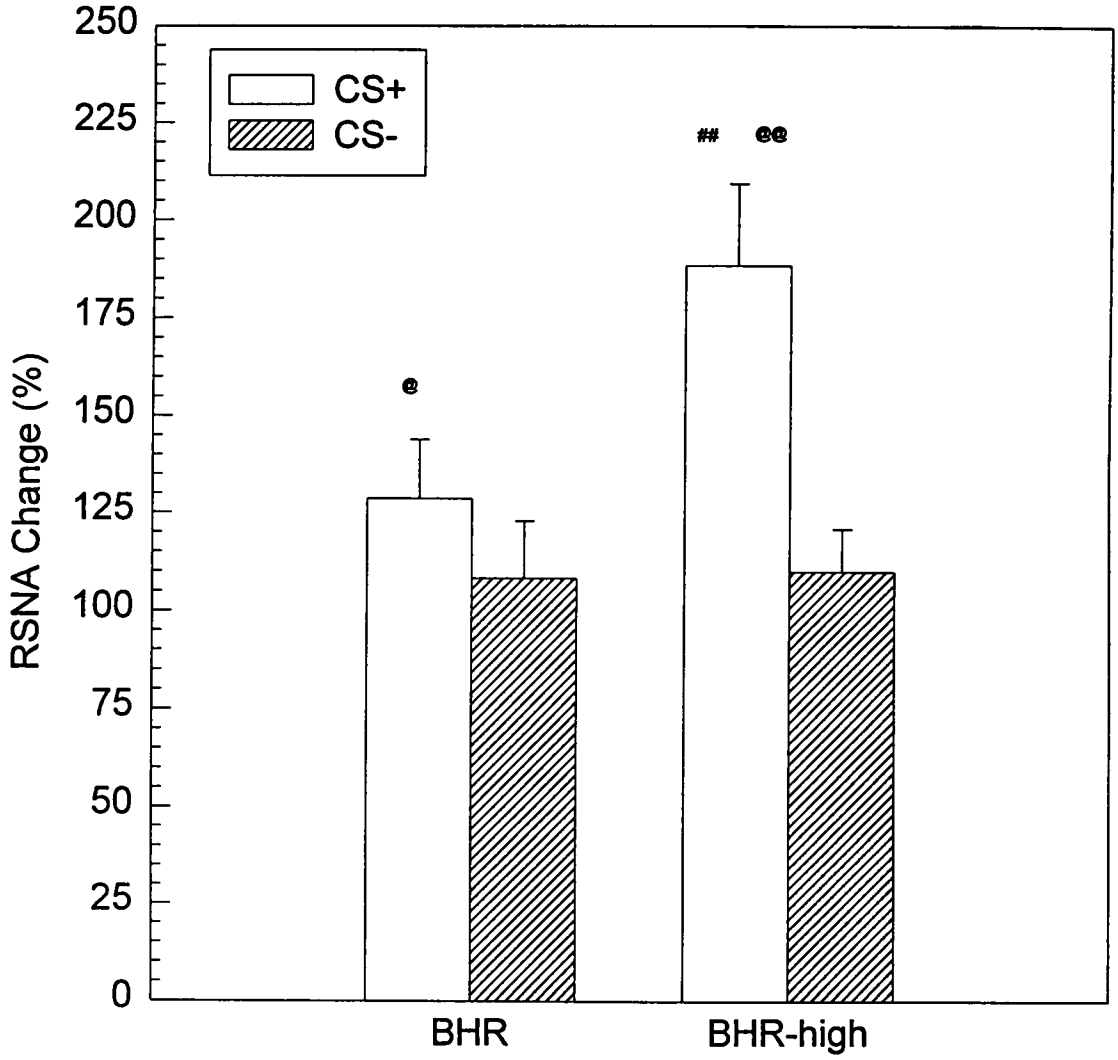

Figure 26. RSNA sudden burst changes from baseline for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. ## p<.01 compared to BHR; @ p<.05, @@ p<.01 compared to CS- within groups.

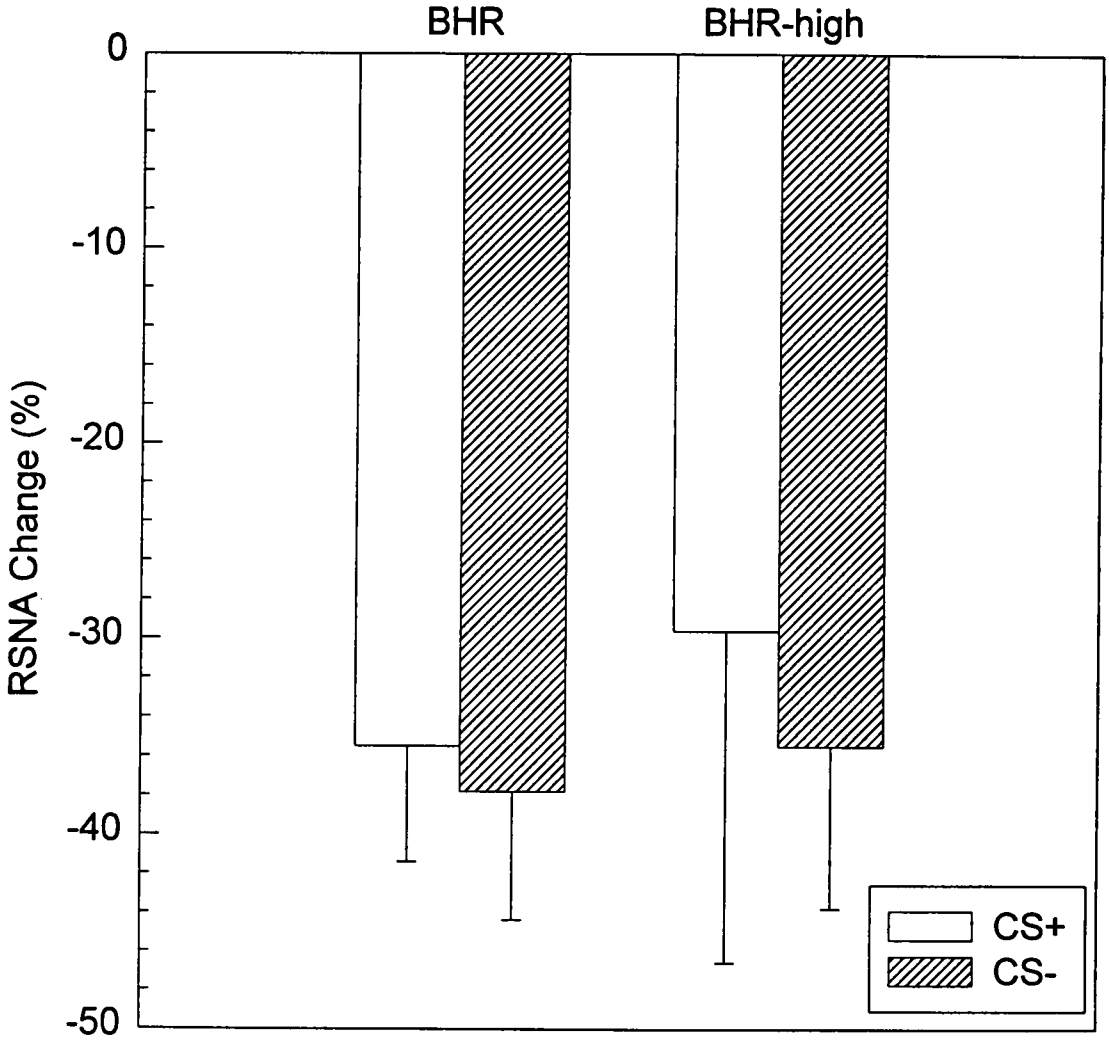

Figure 27. RSNA changes from baseline during quiet period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. T-tests: n.s. between groups or trial types.

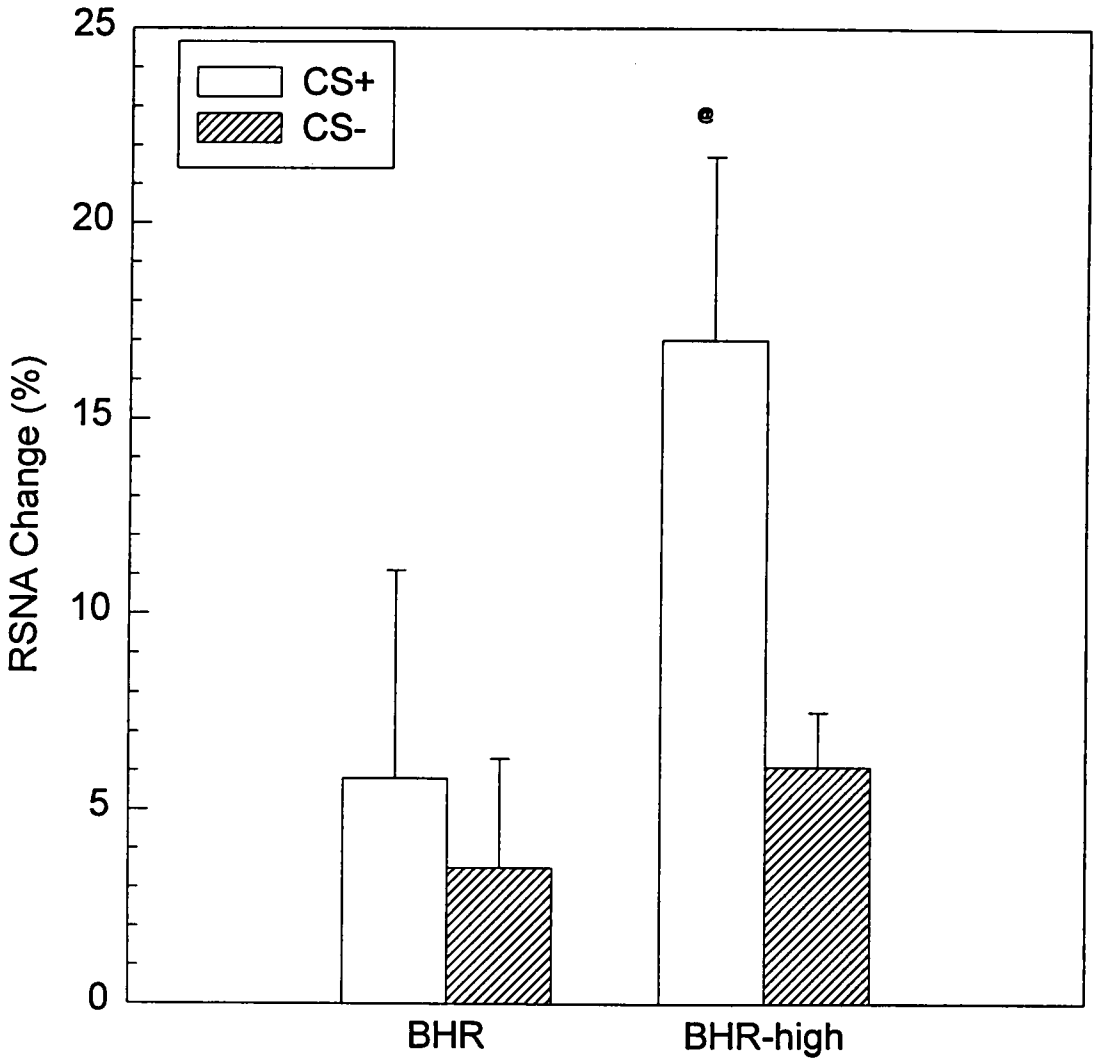

Figure 28. RSNA changes from baseline during tone period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. T-test: p=.13 between groups during CS+; • p<.05 compared to CS- within groups.

BHR and BHR-high groups during the tone period (sec 15.00-23.99) are illustrated in Figure 28. The increase of RSNA during the tone period in BHR-high (17±5%) was approximately three times larger than BHR (6±5%) ($p=.13$) during CS+ trials. However, within BHR-high, a greater ($p<.05$) RSNA increase over baseline was found during CS+ (17±5%) compared with CS- (6±1%) trials.

Heart Rate Responses in BHR and BHR-high

The HR results for BHR and BHR-high groups are described in Table 7 and illustrated in Figures 29-32. The change in HR during various periods is expressed as an increase or decrease from baseline in beats per minute.

As shown in Figure 29, there were no significant differences in baseline HR between the two groups (380±12 bpm in BHR, 391±9 bpm in BHR-high). The first conditioned response in HR change is shown in Figure 30. Both groups had 25-35 bpm increases in HR over their baseline during CS+ and CS- trials. However, there were no significant differences in the magnitude of change between groups or trial types. Figure 31 depicts the bradycardic response following the

Table 7. HR Responses (Mean±S.E.) for BHR and BHR-high during CS+ and CS- Trials

	BHR (N=12)		BHR-high (N=12)	
Variable	CS+	CS-	CS+	CS-
HR_{rest} (bpm)	380±12	380±12	391±9	368±9
ΔHR_{c1} (bpm)	26±9	27±6	32±8	25±4
ΔHR_{qp} (bpm)	-23±5	-23±5	-30±4	-30±5
ΔHR_{k-1} (bpm)	49±5	50±5	63±9	54±5
ΔHR_{tone} (bpm)	-21±4	-6±2	-19±3	-7±2
ΔHR_{uc} (bpm)	-15±6	n.a.	-11±4	n.a.

HR_{rest}: baseline heart rate;
ΔHR_{c1}: Peak HR over baseline at first conditioned response;
ΔHR_{qp}: HR change (drop) from baseline following c1;
ΔHR_{k-1}: HR change from peak to bottom;
ΔHR_{tone}: HR change from baseline during tone period;
ΔHR_{uc}: HR change during unconditioned response period;
n.a.: not applicable (no UC for CS- trials).

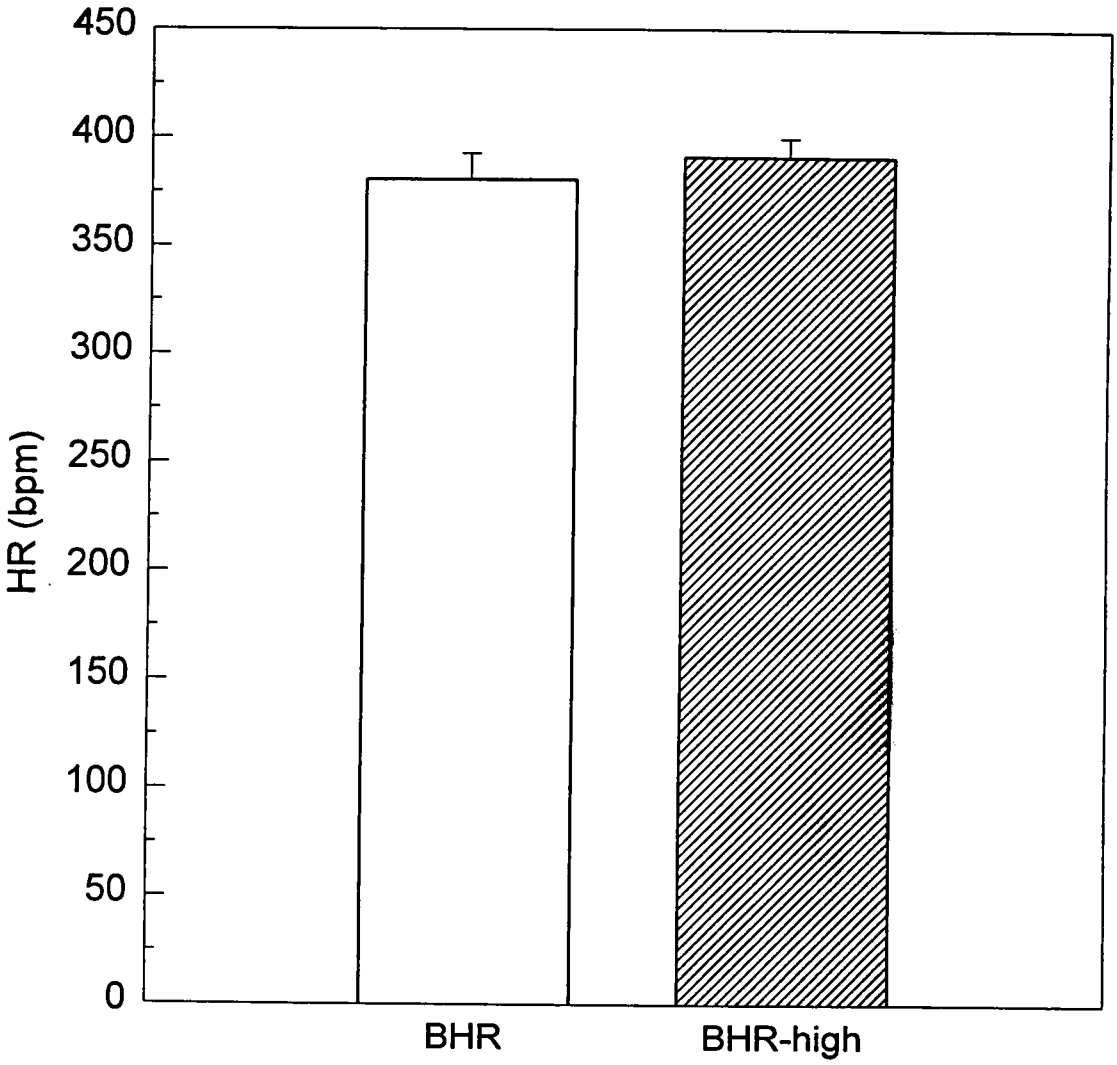

Figure 29. Baseline HR for BHR on normal (BHR) vs. high (BHR-high) salt diet. T-test: n.s. between groups.

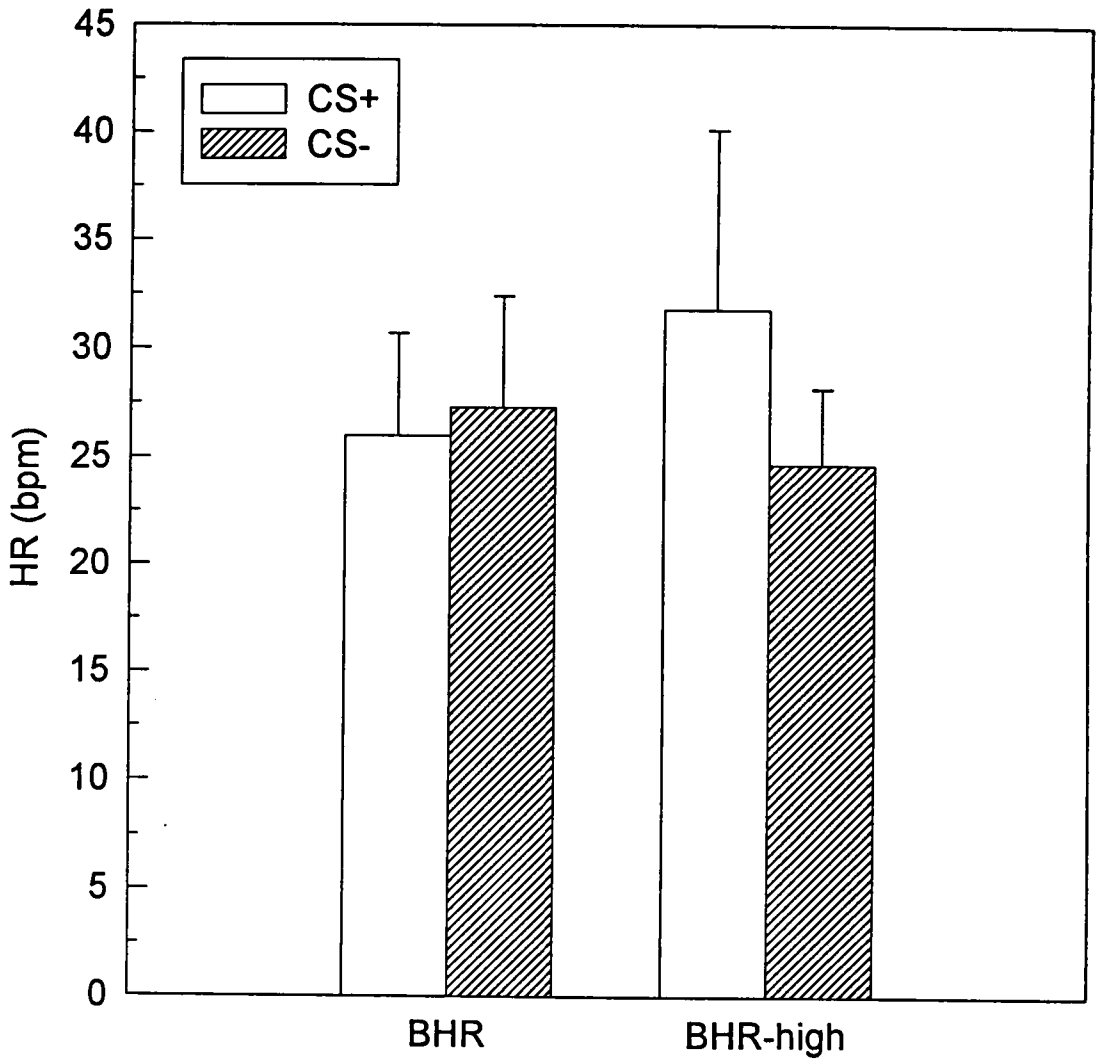

Figure 30. First conditioned HR changes from baseline in BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. T-test: n.s. between groups or trial types.

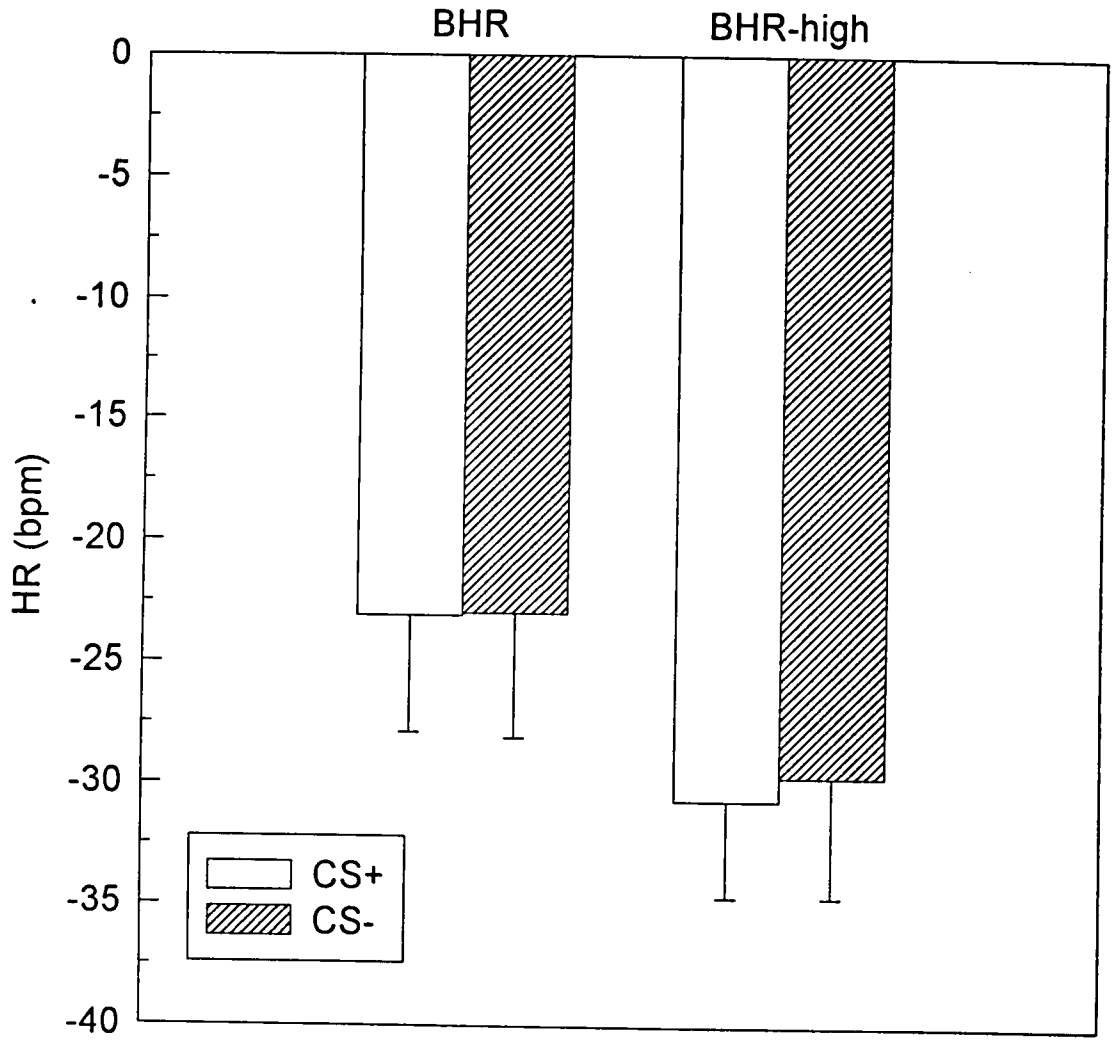

Figure 31. HR changes from baseline during bradycardic response period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. T-test: n.s. between groups and trial types.

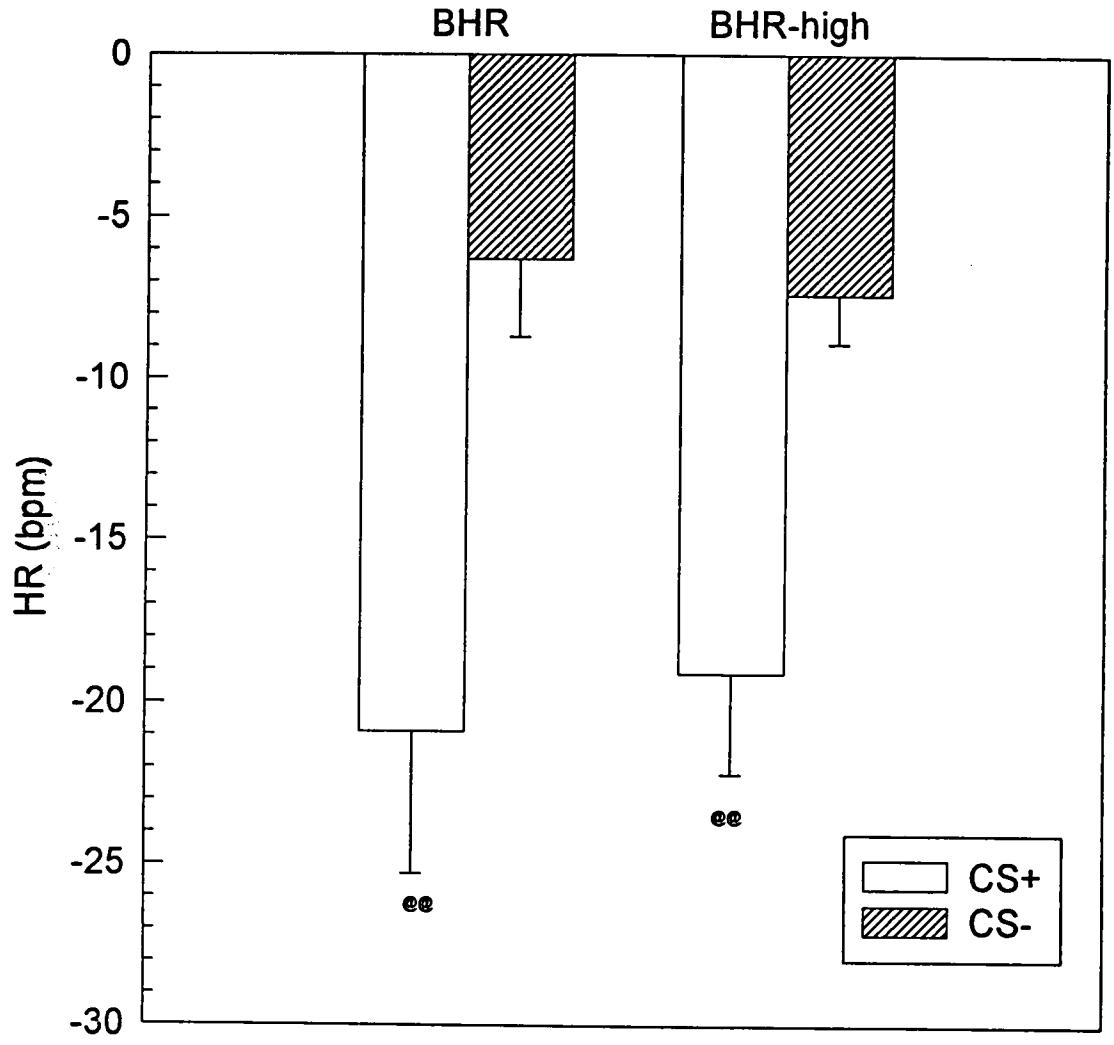

Figure 32. HR changes from baseline during tone period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. @@ $p<.01$ compared to CS- within groups.

first conditioned response. The HR in BHR decreased about 23 bpm during CS+ and CS- trials compared with a decrease of about 30 bpm in BHR-high in both trial types. However, no significant differences in the magnitude were found between groups and trial types. Similar patterns of bradycardia were observed during the tone period in BHR and BHR-high animals. This is illustrated in Figure 32. HR in BHR decreased an average of 21 bpm during CS+ vs. 6 bpm during CS- trials, compared to 19 bpm during CS+ and 7 bpm during CS- trials in BHR-high group. The bradycardic response was greater during CS+ than during CS- trials in BHR and BHR-high groups ($p<.01$ in both groups). However, there were no significant differences between groups for either trial type.

Mean Arterial Pressure Responses in BHR and BHR-high

The MAP results for BHR and BHR-high groups are described in Table 8 and illustrated in Figures 33-37. The change in MAP during various periods is expressed as an increase or decrease from the baseline in mmHg.

The baseline MAP of BHR and BHR-high is shown in

Table 8. MAP Responses (Mean±S.E.) for BHR and BHR-high during CS+ and CS- Trials

	BHR (N=12)		BHR-high (N=12)	
Variable	CS+	CS-	CS+	CS-
MAP_{rest} (mmHg)	132±2	132±2	138±2	138±2
ΔMAP_{c1} (mmHg)	5±1	4±1	8±1	4±1
ΔMAP_{qp} (mmHg)	1±1	-2±1	-1±1	-4±0
ΔMAP_{tone} (mmHg)	4±1	1±0	5±1	1±0
ΔMAP_{uc} (mmHg)	15±2	n.a.	15±1	n.a.
ΔMAP_{post} (mmHg)	-4±1	n.a.	-4±1	n.a.

MAPrest: baseline mean arterial pressure (MAP);
ΔMAP_{c1}: first conditioned (C1) response over baseline;
ΔMAP_{qp}: MAP drop from baseline after C1 response;
ΔMAP_{tone}: MAP change from baseline during tone period;
ΔMAP_{uc}: peak unconditioned MAP response to shock over baseline;
ΔMAP_{post}: MAP depressor response following UC.
n.a.: not applicable (no UC during CS- trials).

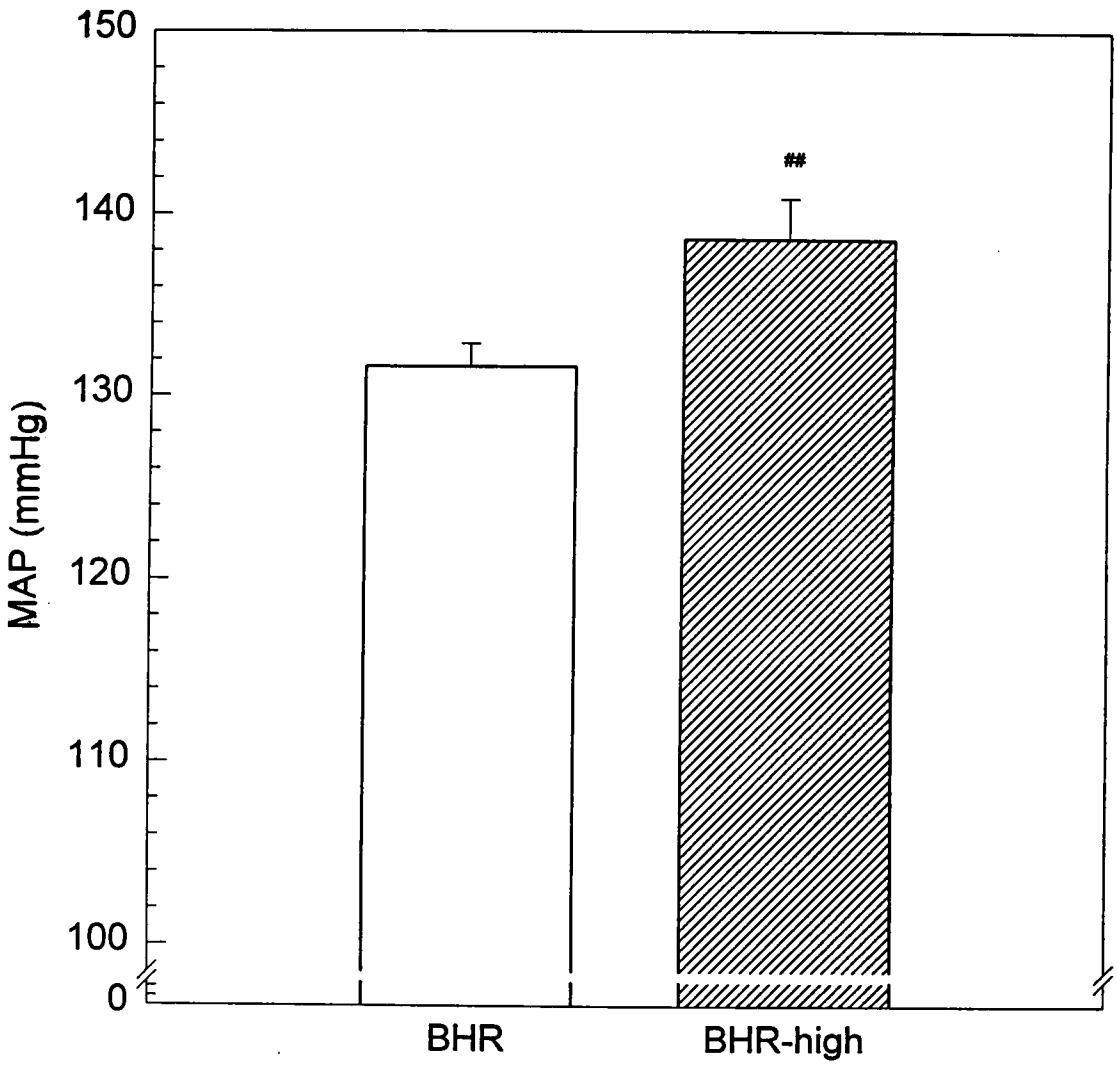

Figure 33. Baseline MAP for BHR on normal (BHR) vs. high (BHR-high) salt diet. ## p<.01 compared to BHR.

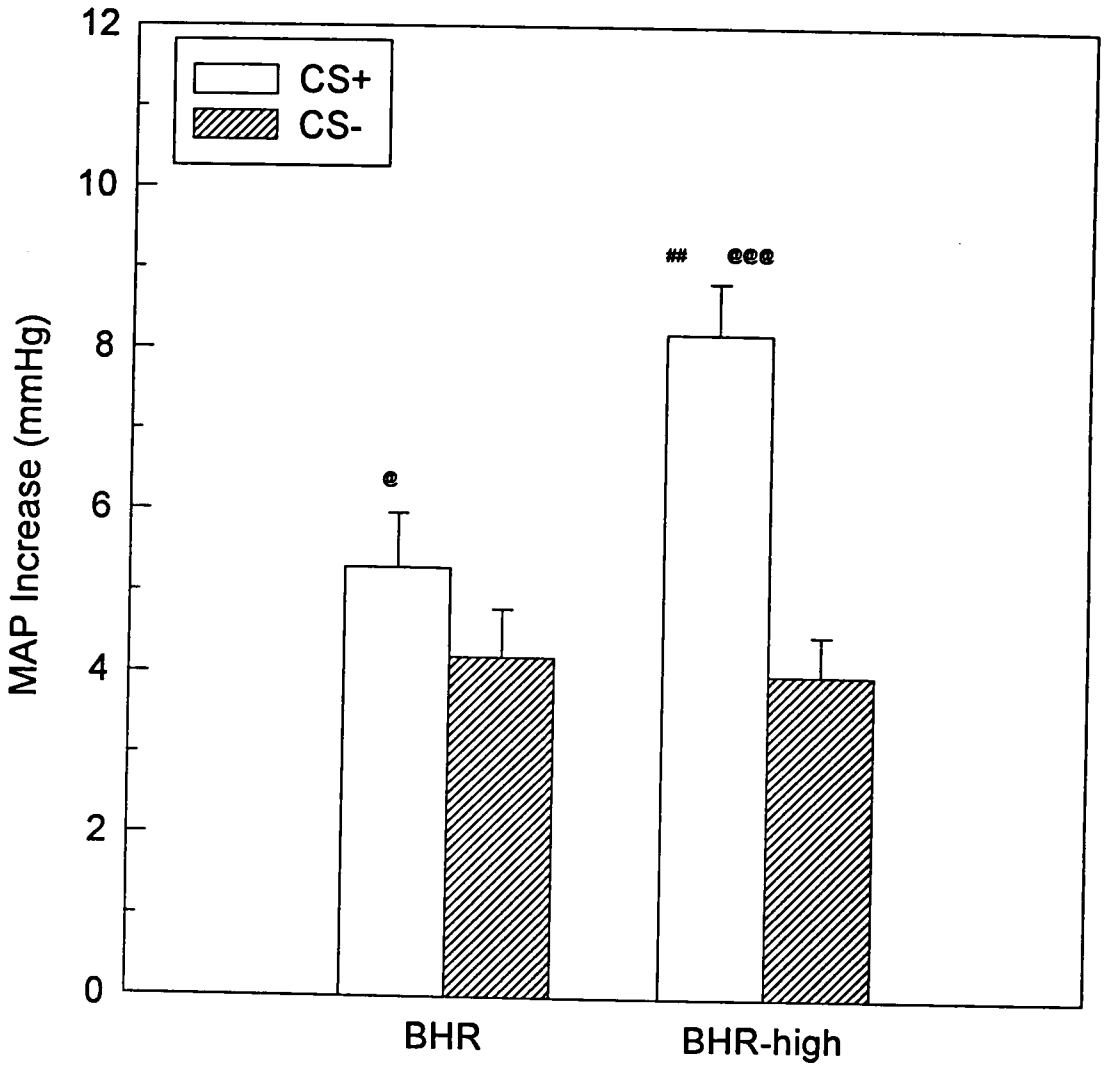

Figure 34. MAP changes from baseline during first conditioned response period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. ## $p<.01$ compared to BHR; @ $p<.05$, @@@ $p<.001$ compared to CS- within groups.

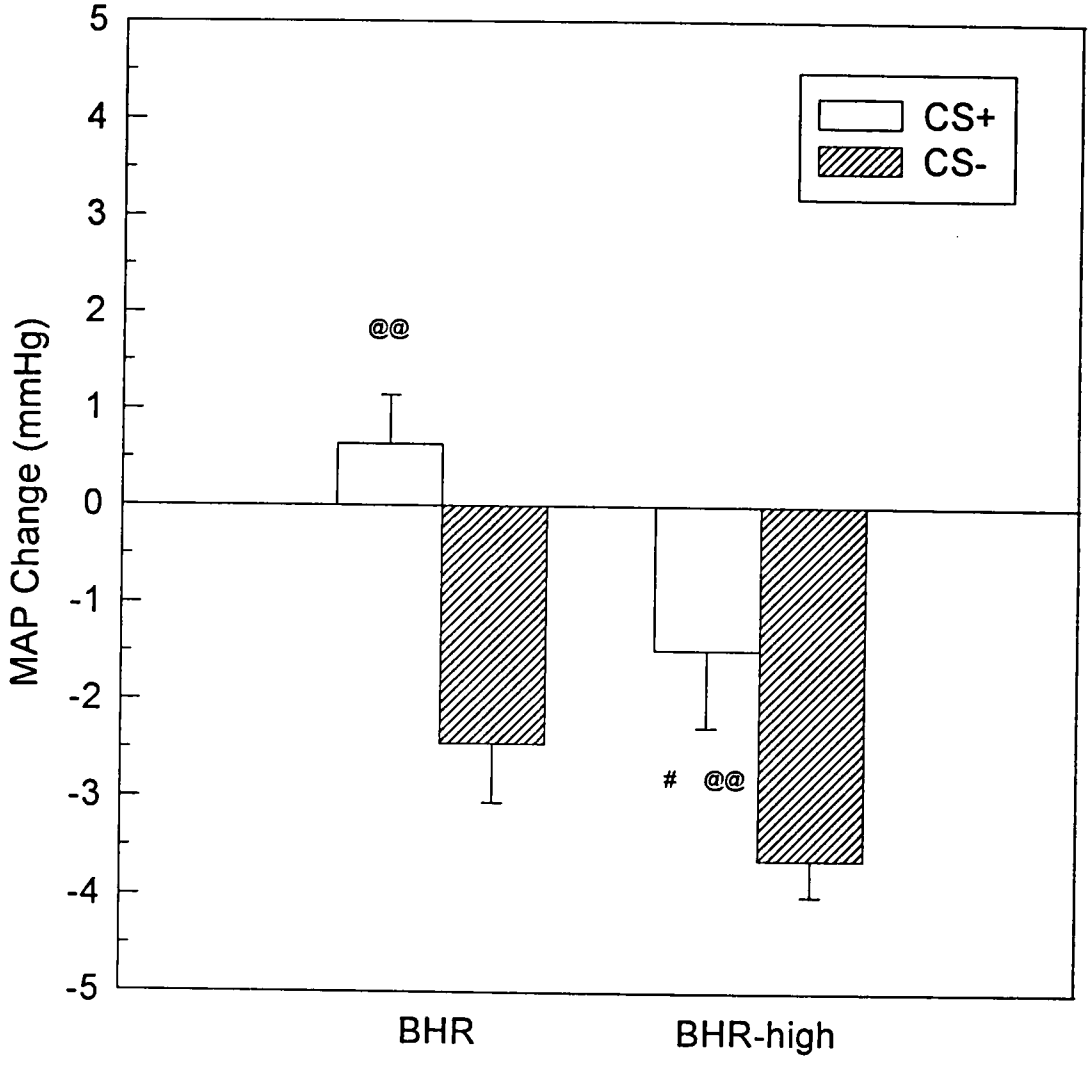

Figure 35. MAP changes from baseline during depressor response period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. # $p<.05$ compared to BHR; @@ $p<.01$ compared to CS- within groups.

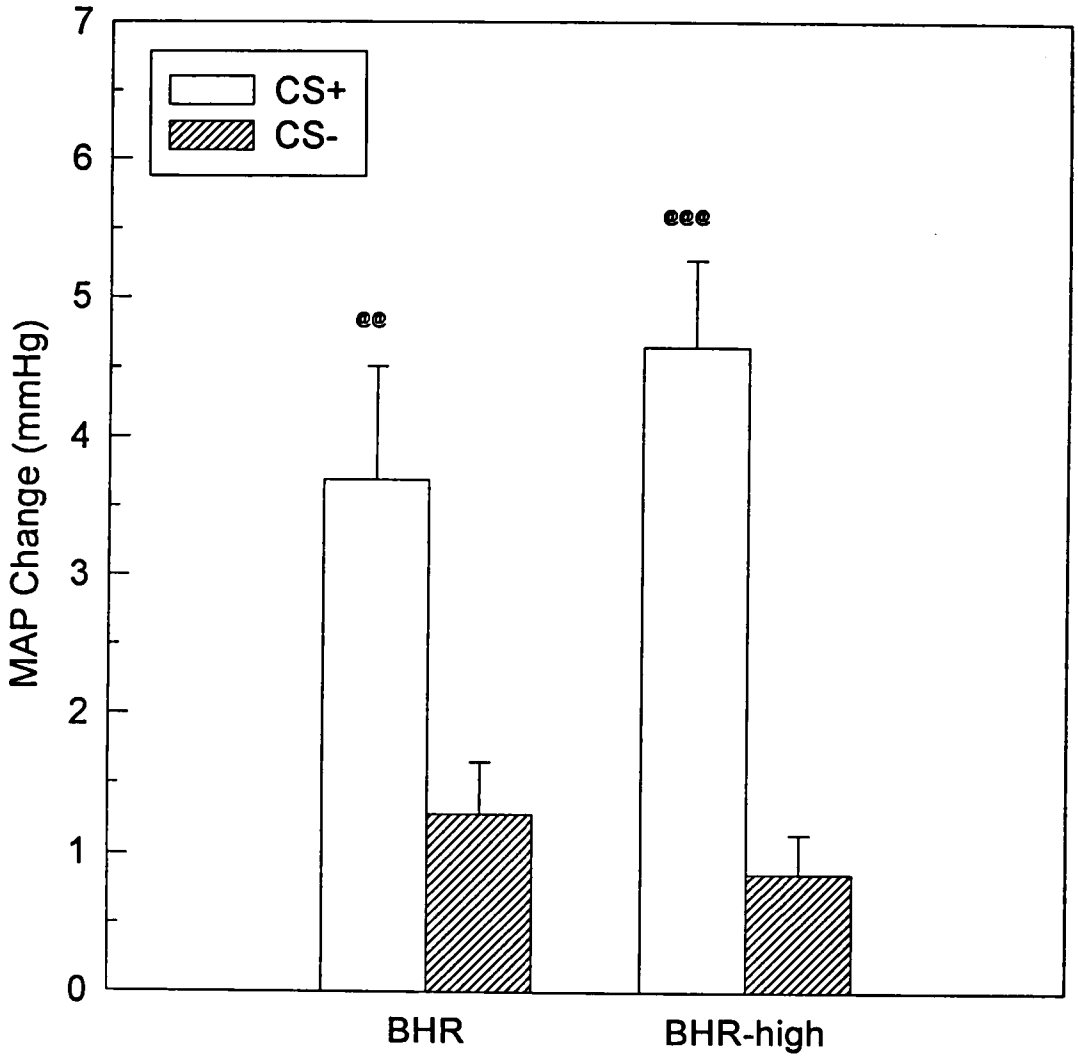

Figure 36. MAP changes from baseline during tone period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ and CS- trials. @@ $p<.01$, @@@ $p<.001$ compared to CS- within groups.

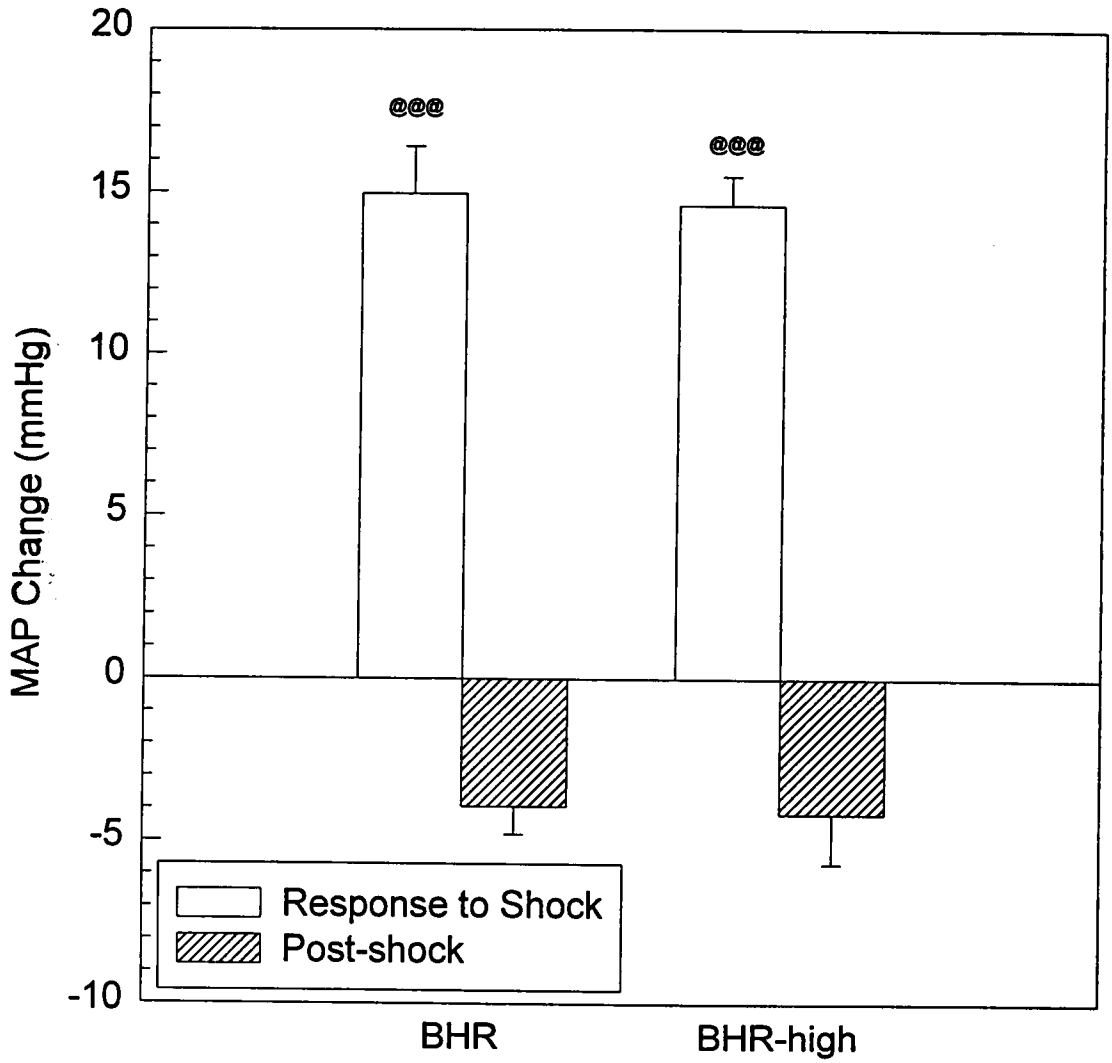

Figure 37. Peak MAP changes from baseline in response to tail-shock and during post-shock period for BHR on normal (BHR) vs. high (BHR-high) salt diet during CS+ trials.
@@@ p<.001 compared to post-shock period.

Figure 33. Animals developed a significantly higher ($p<.01$) baseline MAP on high salt diet (BHR-high: 138±2 mmHg) than on normal diet (BHR: 132±2 mmHg). Results of the first conditioned response in MAP are shown in Figure 34. The measurement is the average during the approximately 1.4 sec of the C1 period. A greater pressor response was observed during CS+ trials (8±1 mmHg) than during CS- trials (4±1 mmHg) in the BHR-high group ($p<.001$). The differences between trial types, though only about 1.5 mmHg in BHR, were significant ($p<.05$).

Figure 35 shows the depressor responses following the first conditioned pressor response. A significantly greater ($p<.01$) depressor response was observed during CS- trials (-2±1 bpm in BHR; -4±0 bpm in BHR-high) compared to CS+ trials (1±1 bpm in BHR; -1±1 bpm in BHR-high) in each group. However, group differences were found significant only during CS+ trials ($p<.05$). Figure 36 shows the pressor response during the tone period (sec 15.00-23.99). Both BHR and BHR-high had greater MAP increase during CS+ (5±1 mmHg in BHR-high, 4±1 mmHg in BHR) than during CS- (1±0 mmHg in both groups) trials ($p<.001$ in BHR-high, and $p<.01$ in BHR

between CS+ and CS- trials). However, the difference between groups were not significant during CS+ and CS- trials.

Figure 37 shows the unconditioned response to tail-shock and the post-shock depressor response. Similar MAP changes were observed in BHR and BHR-high groups in shock and post-shock responses. Both BHR and BHR-high groups had a 15 mmHg MAP increase in response to tail-shock followed by a significant ($p<.001$) depressor response (4 mmHg below baseline or 19 mmHg below the peak pressor response).

CHAPTER IV

DISCUSSION

In this chapter, the results from two experiments will be discussed. The first experiment studied genetic influences on RSNA, HR and MAP responses to a classical discriminative conditioning paradigm in conscious WKY, BHR and SHR. The second experiment studied the effect of high salt diet on the physiological response to behavioral stress in BHR. But first, the general pattern of neural and cardiovascular responses to behavioral stress will be discussed. Finally, weaknesses in this study and suggestions for future study will be addressed.

I. THE PATTERN OF NEURAL AND CARDIOVASCULAR RESPONSES TO BEHAVIORAL STRESS

As mentioned previously, in this study, behavioral stress was administered in a revised Pavlovian conditioning paradigm (Cohen and Randall, 1984). As demonstrated in the pilot study, there is no evidence suggesting any differences

in psychological properties between the pulsed and non-pulsed tones. They cause comparable MAP startle responses and, as conditioning stimuli, can easily be discriminated.

Thus, although the evoked physiological responses to CS+ in the present study involve a component of startle, its salience can be revealed by comparing it to the responses elicited by the CS- signal. Therefore, any differences in sudden burst activity between CS+ and CS- must be due to the fact that the rat has learned to associate shock with only one of these tones, the CS+.

Since the sudden burst RSNA response is a more direct index of central processing than is HR or MAP (both of which are affected by other variables such as hormones, receptor density, intrinsic mechanisms, etc.), it may be that the sudden burst RSNA response is one of the best indices yet developed for assessing neural reactivity to stress. Inherent differences in this reactivity may, in turn, be an informative link between stress and hypertension. This issue will be addressed in a subsequent section.

Pattern of RSNA, HR and MAP Responses to Discriminative Conditioning

Feedback is a fundamental mechanism for all biological control systems, including blood pressure regulation. The short-term regulation of blood pressure is directly controlled by the sympathetic and parasympathetic nervous systems via the baroreflex and several other feedback systems. These regulatory systems can maintain blood pressure in a very narrow range in response to abrupt changes caused by internal or external events (e.g., orthostasis, exercise, stress). This is called a closed-loop control system. This may explain the bradycardic and depressor responses following the pressor conditioned response (C1 in Figure 2) in this study. These responses may have been caused by increased parasympathetic and decreased sympathetic activities as a result of the baroreflex.

On the other hand, sensory events (e.g., stress signals) can also control motor activity (stress responses) more effectively by providing advance rather than feedback information. Advanced information can then be used to adjust the controlled variables before events occur that would

influence them. This process is an example of a feed forward or open-loop control system. The fact that the abrupt, synchronized postganglionic sympathetic discharge (sudden burst) lasted only about a half sec in all groups and ceased even before the first conditioned MAP reached its peak suggests that it is an open-loop response. This distinctive component of the RSNA response is the first part of the learned response to the expected tail-shock. The onset latency of this response to CS+ was identical in all three strains (.16±.01 sec). Similar onset latency results (.16 ±.03 sec) have been reported in adult Sprague-Dawley rats using the same protocol as that used in this study (Randall et al, 1995). This latency would appear to represent the duration from perceiving and analyzing the acoustic signal (tone) to conducting sympathetic discharges to the renal nerves.

The quiet neural traffic period is probably a result of the baroreflex though it started even before the first conditioned MAP response reached its peak. However, we can not make this conclusion without excluding two other possibilities. First, the mechanism of the quiet period in

RSNA following the sudden burst RSNA has not been investigated in this or any other study. It may have been caused by either a temporary exhaustion of central or peripheral neurotransmitters, or an inhibition resulting from feedback processes such as the baroreflex. The latter possibility is, of course, in contradiction to the open-loop hypothesis. This possibility may be tested in combination with molecular dialysis techniques. These are technically difficult studies, especially in the conscious, freely-moving organism.

Second, as seen consistently in all groups, abrupt HR responses started shortly after the onset of sudden burst RSNA, but reversed before the sudden burst RSNA reached its peak. Whether the RSNA is parallel to the animal's overall and/or cardiac sympathetic activity will be discussed in a subsequent section. Direct analysis of parasympathetic control of cardiovascular function over such small intervals has not been reported in the literature. However, no matter whether the sympathetic responses are parallel in both renal and cardiac sympathetic nerves or not, the bradycardic response during the increasing phase of sudden burst RSNA

(when sympathetic activity is high) can only be explained as the effect of an increased parasympathetic activity. Thus, this response can hardly be classified as open-loop control. Rather, we propose that this is an "early" feedback response mainly under parasympathetic control, occurring before either RSNA or MAP reaches its peak. The major implication of this conclusion is that autonomic nervous control system has an early detection or prediction (in less than .10 sec) function, and a rapid correction capability for blood pressure regulation. Thus, a closed-loop control system (specifically, the parasympathetic system) can be activated even before the open-loop sudden burst response is terminated, or in less than .10 sec following the tone onset. Again, future studies are needed to improve our understanding of autonomic nervous system control mechanisms.

The diameter of nerve fibers in cardiac and renal branches of the sympathetic nervous system is not available in the literature. However, both branches are type C unmyelinated fibers with a conducting velocity of .5-2 meters/sec. Since the fibers to the heart travel a shorter

distance than those to the kidneys, the total time for sympathetic signals to reach the heart is probably shorter than to the kidneys.

The first conditioned response in MAP started at .4 sec from the onset of the tone or .24 sec from the onset of the RSNA sudden burst. Thus, it appears that the .24 sec duration represents the time needed from the postganglionic sympathetic discharge to the reaction of the cardiovascular effector, such as cardiac muscle or blood vessel smooth muscle. It appears that the abrupt conditioned MAP response in this protocol was caused directly by a sudden burst of sympathetic output in response to the CS+ signal, or to a startle response in the case of CS- trials. Randall (1995) likened this MAP response to "a ballistic response such as occurs after a bat briefly contacts a baseball: the subsequent flight of the ball is determined at the brief moment of contact" (p. 1246). The magnitude of the first conditioned MAP response was significantly correlated ($r=.4062$, $p<.05$) with the magnitude of the RSNA sudden burst. However, the relative contribution of cardiac output vs. peripheral resistance to this MAP response is yet to be

clarified. Nevertheless, these data suggest that HR was not a supporting factor since it decreased during the conditioned MAP response period compared to baseline. Thus, it can only be caused by increased stroke volume if there was an increase in cardiac output. Alternatively, total peripheral resistance may have increased.

The onset latency for the RSNA sudden burst was .04-.05 sec shorter in CS- than in CS+ trials in all groups. There are two possible mechanisms that could cause such a difference: the first is that the time required to analyze a non-pulsed signal is shorter than that required for a pulsed tone; the other is that the interruption of a pulsed tone caused a longer latency time during CS+ trials.

The following quiet neural traffic period was also coupled with a below baseline depressor response in MAP. The depressor response may have been caused by a baroreflex response which in turn activated the parasympathetic system and decreased sympathetic activity. However, as mentioned above, it is not clear whether the quiet neural traffic period was caused by exhaustion of neural transmitters, loss

of receptor affinity, or temporary shut down of sympathetic system activity caused by the baroreflex.

During the rest of the tone period (sec 15.00-23.99), steady increases in RSNA and MAP were coupled with a gradual bradycardic response during CS+ trials, whereas there was a much smaller bradycardia in SHR and little HR change in WKY and BHR during CS- trials. The continuous bradycardia in the tone period suggests that the parasympathetic nervous system was activated above baseline, but not sufficiently to bring MAP to baseline levels. Also, the continuation of the stress signal during CS+ trials caused a temporary shift of the blood pressure set-point to higher levels. This finding is important in that it highlights the role of the sympathetic nervous system in preparing the organism for the fight or flight response to a stressful stimulus. Another interesting conclusion from these observations is that, either because of the limitation of parasympathetic activity or the shift of blood pressure set-point, sympathetic activity is dominant over parasympathetic activity in the sustained stress condition. Differences between groups and trial types will be discussed in subsequent sections.

Though not formally pursued in this experiment, plasma epinephrine and norepinephrine levels may have increased and contributed to a higher peripheral resistance during the sustained blood pressure elevation period. This could partially explain the blood pressure elevation while HR was decreased. There is evidence from other studies to support this notion. For example, plasma epinephrine and norepinephrine levels were found to be two times higher during an aversive classical conditioning paradigm (tone, light and tail-shock) in BHR compared to baseline. Similar changes were found in WKY but to a smaller degree (Hubbard et al, 1986).

II. INFLUENCE OF GENETIC PREDISPOSITION

The baseline data suggest that genetic predisposition contributed differently in the three groups only with respect to arterial pressure, and not with respect to HR or RSNA. It is not surprising, however, that there were no significant differences in HR among the three groups, since HR has never been shown to be a determining factor of blood pressure in humans or any animal model. The real

physiological importance of resting HR, if any, has yet to be understood. On the other hand, the results for baseline RSNA deserve more discussion.

Renal Sympathetic Tone

Although the importance of both tonic (baseline) and phasic (reactivity) sympathetic nervous system activity in the regulation of cardiovascular function and the development of hypertension has been generally appreciated, the matter of renal nerve activity measurement needs to be dissected for better understanding. First of all, nervous connections between the kidneys and the celiac plexus, major and minor splanchnic nerves, and several other nerves have great individual variability or specificity in rats (Drukker et al, 1987) as well as in humans (Mitchell, 1950). There are several, instead of one, renal nerves (branches) surrounding the renal artery and vein which enter each kidney. The number of major branches and their position relative to the renal artery differ from rat to rat. In the present study, the largest renal nerve (or branch) was chosen for the implantation of recording electrodes. Baseline RSNA is the synchronized discharge of the fibers in

the compound nerve. Therefore, the magnitude of the baseline RSNA is determined by the number and property of the fibers in that nerve which vary from one animal to another. This may explain the large variance in baseline RSNA both within and between strains.

A second consideration is whether RSNA is proportional to the animal's overall sympathetic activity. There is indirect evidence for such a hypothesis. For example, renal nerves are predominantly noradrenergic fibers and there is no cholinergic innervation in kidney, so only sympathetic activity is being measured (Barajas et al, 1992); the intensity or frequency of renal nerve stimulation is positively related to the major renal functions (DiBona, 1985); and in the present study, a significant positive correlation ($r=.6424$, $p<.001$) was found between RSNA change and MAP increase during the tone period for CS+ trials as well as between the sudden burst RSNA and the first conditioned MAP response ($r=.4061$, $p<.05$).

However, there are data that argue against sympathetic nerve activity from one bed serving to index the whole

sympathetic system. For example, 25 healthy young male students (20-23 years old) were tested to evaluate the relationship between myocardial and peripheral vascular responses during different stressors. It was reported that the correlation between cardiac output and forearm blood flow change was significant for the reaction time task, but not for the mental arithmetic or cold pressor tasks (Allen et al, 1987). This suggests that autonomic nervous system control of different target organs is not always proportional.

The last, but most fundamental, question is whether there are significant differences in baseline RSNA among SHR, BHR and WKY. Higher baseline MAP in SHR compared to BHR and WKY could be maintained by either a higher sympathetic tone or baseline (number of nerve fibers firing and neurotransmitters released during a specific time period), or a defect in target organs (higher receptor sensitivity and/or other vascular defects which increase muscle excitability or contractility). Direct, reliable comparisons of compound sympathetic activity (e.g., RSNA) between animal strains are not yet technically feasible. Thus, a direct

answer to the last question is not yet available.

However, a large number of observations in various animal models of hypertension support both the role of sympathetic tone and target organ defects in hypertension (Abboud, 1982). Indeed, evidence supports the conclusion that enhanced sympathetic tone contributes, at least in part, to the maintenance of MAP in some animal models. For example, lesions of several brain structures such as the nucleus tractus solitarius and the anteroventral area of the third ventricle reduce sympathetic tone as well as MAP (Carey et al, 1979; Sanders et al, 1989b). Furthermore, a reduction in baseline sympathetic activity elicited by many antihypertensive drugs can correct the hypertension in the majority of clinical and experimental situations regardless of their primary cause.

In summary, RSNA is an excellent measure of sympathetic nervous system activity to the kidney. However, there are occasions during which it may be an index of overall sympathetic drive. Finally, the technique cannot yet be used to assess strain differences in baseline RSNA. However,

changes in RSNA between strains can reliablbly be compared. We turn now to the discussion of reactivity differences in RSNA between groups and trial types.

Sympathetic and Cardiovascular Reactivity

We measured reactivity as the sudden burst RSNA and the first conditioned MAP and HR responses to behavioral stress signaled by tone onset. Results revealed significant differences among the three strains with different genetic predispositions in all dependent measurements. Results of the sudden burst RSNA changes (177% in SHR, 129% in BHR, and 105% in WKY in CS+ trials) suggest that genetic predisposition determines reactivity in sympathetic discharge during behavioral stress. Discrimination between CS+ and CS- trials was clearly evident. Some structural and functional differences may exist in brainstem nuclei (e.g., ventral lateral medulla and nucleus tractus solitarius) where the basic autonomic nervous system center is located. Moreover, higher brain centers (e.g., the anteroventral area of the third ventricle and hypothalamus) that have inputs to this final common pathway may also be involved, since these centers are involved in perceiving and analyzing the tone

signals and/or determining their emotional import.

The significant correlations between sudden burst RSNA and both MAP and HR reactivity suggest that these latter responses were, at least partially, directly determined by the sudden burst in sympathetic discharge. It is also important to realize that the sensitivity of receptors in target tissues (cardiac and vascular smooth muscles), as well as muscular excitability and contractility, are also important factors in blood pressure regulation (Allen et al, 1987; Brown et al, 1981; Oparil et al, 1987). Indeed, differences in such factors in the three animal strains were suggested by the results of comparisons of the sudden burst to MAP reactivity ratio, as shown in Table 3. This ratio represents the percentage change over baseline in RSNA required to increase MAP by 1 mmHg. Therefore, a smaller ratio, like that seen in SHR in comparison to WKY and BHR, suggests a greater number of receptors, higher sensitivity and/or vascular contractility. This is consistent with Lee's finding (1985) of a higher nerve density in the large mesenteric arteries and a greater number of smooth muscle layers in small blood vessels of SHR compared to WKY during

the prehypertensive phase. Also, fewer type 2 renal chemoreceptors were reported in old SHR, while WKY rats have only the type 2 chemoreceptors and no type 1 chemoreceptors (Recordati et al, 1980).

Differences between groups in MAP and HR reactivity during CS+ trials were significant. SHR had a higher tachycardia and greater pressor response in CS+ trials. Interestingly, there were no significant differences between BHR and WKY. Similar observations were recently reported for RSNA, HR and MAP responses to air jet stress in WKY and BHR on a normal salt diet (DiBona, 1995). These results suggest that the BHR is essentially normotensive unless subjected to chronic stress or high salt diet.

It is so far not clear what fundamental genetic differences exist among the three strains. Based on an analysis of DNA "fingerprint" patterns generated with six multilocus probes, it was reported that the SHR and WKY (from Charles River Laboratories, Inc.) only shared 50% of their DNA fingerprint bands in common (Lezin et al, 1992). Also, Johnson et al (1992) reported an extremely high amount

of nucleotide divergence (1 change per 62 base pairs) between SHR and WKY. This is comparable to the maximum divergence possible between unrelated humans given the fact that these two strains have been derived from the same origin for only 35 years. To what extent this disparity carries over to genes controlling blood pressure is not known.

With respect to such large genetic differences between SHR and WKY and the small number of genes believed to be involved in hypertension, as well as the uncertainty of gene cosegregation, the fundamental genetic predisposition of BHR may be hard to predict. In other words, the smaller the number of genes involved in hypertension, the greater the standard deviation in blood pressure and reactivity among BHR individuals will be. On the other hand, the genetic predisposition of BHR will be closer to the midpoint of SHR and WKY if a fairly large number of hypertensive genes are involved and evenly cosegregated. Problems that need to be addressed in the future include the identification of the principal hypertensive "genes," if any, the pattern(s) of cosegregation among these and other genes, and the influence

of environmental factors on the expression of these genes.

Baroreflex Gain

The RSNA, HR and MAP changes and their interaction, suggestive of baroreflex control, have not previously been reported in the conscious animal literature. During the quiet neural traffic period following the sudden burst, RSNA in all strains decreased remarkably from the sudden burst peak to well below baseline in CS- trials (-18% in WKY, -38% in BHR, -29% in BHR). However, in CS+ trials, SHR could decrease to only slightly above its rest level (2%), while BHR and WKY had decreases comparable to their CS- trials (-13% in WKY and -36% in BHR). The inability of SHR to reverse the renal nerve response during CS+ trials compared to the other two strains suggests that the SHR's total gain of baroreflex in terms of sympathetic activity decrease was diminished.

As a result of this abrupt baroreflex input, HR decreased 87 bpm from its peak in SHR during CS+ trials, while it decreased only 50-60 bpm in both trial types in BHR and WKY as well as during CS- trials in SHR. This greater

bradycardic response in SHR was probably caused by a greater parasympathetic discharge to the heart since its change in sympathetic discharge was higher than other two strains.

In both trial types for the three strains, MAP decreased virtually to baseline. SHR had no less baroreflex gain in MAP in each trial types than WKY or BHR. This observation may provide some insight into the balance between sympathetic and parasympathetic nervous system responses during stress. If the baroreflex gain in MAP is solely manipulated by the two branches of the autonomic nervous system, then all animal strains reached their pre-stress (baseline) MAP levels, but utilized different autonomic control mechanisms to reach that level. For example, the MAP return to baseline in SHR during CS+ trials was accomplished through a slightly above baseline level (2%) of sympathetic discharge and above baseline parasympathetic activity (HR 45 bpm below baseline or -87 bpm from the peak HR). On the other hand, MAP decreased to baseline levels in WKY and BHR during CS+ trials through a greater decrease in sympathetic discharge (-13% in WKY and -36% in BHR) combined with a smaller bradycardia (-32 bpm

from baseline or -56 bpm from peak HR in WKY; -23 bpm from baseline or -49 bpm from peak HR in BHR). This suggests that there may be some differences among animal strains in the way their autonomic branches respond to stress.

For the rest of the tone period (sec 15.00-23.99) during CS+ trials, SHR maintained 36% higher RSNA, 15 mmHg higher MAP, and 45 bpm lower HR than baseline. In contrast, significantly smaller bradycardia in the other two strains (-9 bpm in WKY and -21 bpm in BHR) combined with a smaller increase in RSNA (33% in WKY and 28% in BHR) and MAP (1 mmHg in WKY and 4 mmHg in BHR) were observed in CS+ trials. The fact that RSNA was higher than baseline while the HR was still below resting levels suggests that both sympathetic and parasympathetic discharges were higher than baseline levels in all strains. This provides a novel insight into the delicate balance between the two autonomic branches. This observation raises a concern regarding the advisability of the current trend to ignore the importance of parasympathetic, while emphasizing sympathetic, involvement in hypertension.

Another interesting finding during the rest of the tone period (sec 15.00-23.99) during CS+ trials was that significant correlations were found between the increases of RSNA and MAP ($r=.6424$, $p<.001$), as well as between changes of HR and MAP ($r=-.3864$, $p<.05$), but not between changes of RSNA and HR ($r=.0866$). This observation suggests that the pressor response during behavioral stress is caused primarily by increased sympathetic output, while the bradycardic response is the result of baroreflex activation and thus increased parasympathetic discharge. It is not clear why the baroreflex did not shut down the sympathetic output even more instead of increasing the parasympathetic discharge during the tone period. Since the baroreflex can be overridden by the hypothalamus during fight or flight, there may be partial overriding. Two possibilities are worth mentioning here. First, the continuation of the tone may have served as a signal reinforcing the animal's expectation of tail-shock during CS+ trials. Thus, the sustained high sympathetic discharge might be a result of the continued signal. Second, the set point of the baroreflex control system might have shifted to a higher level, which required a higher sympathetic discharge to maintain it. This suggests

that SHR had a greater shift of baroreflex set point during continued stress (sec 15.00-23.99) while its acute total gain (following the first conditioned response) in MAP was the same compared to WKY and BHR.

The unconditioned pressor response to tail-shock was significantly greater in SHR (27 mmHg) compared to WKY (16 mmHg) or BHR (15 mmHg), while the post-shock depressor response was greater in WKY (-10 mmHg) than in BHR (-4 mmHg) or SHR (-2 mmHg). Again, the total baroreflex gain in MAP (from the peak to the bottom) was similar in all three groups. It is interesting to note the unique response of the BHR: their MAP responded to tail-shock to the same extent as WKY, but their depressor response was similar to that seen in SHR.

In summary, results from the first experiment showed that the onset of a tone caused an abrupt sudden burst RSNA response which in turn increased the HR and MAP in about .5 sec. This was followed by a quiet neural traffic period in RSNA and a bradycardic/depressor response which may have resulted from increased parasympathetic and decreased

sympathetic activities through a baroreflex control mechanism. A sustained pressor/bradycardic response and apparently higher sympathetic and parasympathetic activities were observed in all animals, but the magnitude differed depending on the strain and trial type.

Our conclusions about the influences of genetic predisposition are: (1) genetic predisposition determines the baseline MAP, but not HR or RSNA levels; (2) genetic predisposition determines reactivity to stress in RSNA, HR and MAP; (3) the RSNA, MAP and HR responses to the two trial types (CS+ and CS-) can be effectively discriminated and animals can establish behavioral responses to stress through a learning process; (4) SHR may have a higher receptor sensitivity to norepinephrine and epinephrine, and/or vascular defect (e.g., higher contractility) when compared to WKY and BHR; (5) though SHR, BHR and WKY appear to have comparable total baroreflex gain in MAP in response to conditioned and unconditioned stimuli, the set point appears to be shifted differently depending on genetic predisposition, to a higher than baseline level during a continued conditioning signal; (6) sympathetic activity

appears dominant over parasympathetic activity in a sustained stress condition.

III. EFFECT OF HIGH DIETARY SODIUM INTAKE

The effect of high dietary sodium (8% NaCl) intake was tested only in the BHR strain, for reasons including time limits and costs. As mentioned in the introduction, salt-induced hypertension has been reported in animal models such as BHR, SHR, and Dahl salt-sensitive rats (Lawler et al, 1987 & 1993; Mainsail and Drueke, 1992).

Although the importance of renal function (Dahl et al, 1974), sympathetic outflow (Lee et al, 1989; Sanders et al, 1989a), and the role of the renal nerve (Katholi et al, 1983; Lawler et al, 1989; Winternitz et al, 1980) has been studied, direct measurement of the influence of high salt intake on renal nerve activity and its relation to HR and blood pressure during behavioral stress has been poorly documented. One recent study reported that BHR on high salt diet responded to air jet stress with higher frequency, shorter duration and larger height of renal sympathetic

nerve peaks compared to BHR on normal diet (DiBona et al, 1995). Data during different periods of behavioral stress were not available.

In the present study, baseline MAP was significantly higher in BHR on high salt diet than on normal diet (138 mmHg in BHR-high vs. 132 mmHg in BHR). However, there were no differences between the two groups in baseline HR or RSNA. As discussed in a previous section, the relevance of the absolute value of baseline RSNA is limited because of its huge individual variability. Thus, it is not clear whether high salt increased baseline RSNA. Other studies relating high salt diet and baseline levels of sympathetic activity have not been consistent. For example, lesions of brainstem sympathetic control centers (Lee et al, 1989) and the anteroventral area of the third ventricle (Sanders et al, 1989a) prevented salt-induced hypertension in experimental animals. On the other hand, high sodium diet did not change the resting catecholamine levels in Dahl salt-sensitive and resistant rats (McCarty et al, 1982).

The main goal of this experiment was to examine the

influence of high salt diet on the sympathetic nervous and cardiovascular responses to behavioral stress. High salt diet significantly enhanced the RSNA sudden burst response to CS+ (197% increase in BHR-high vs. 129% increase in BHR over baseline), but not to CS- (87% increase in BHR-high vs. 104% increase in BHR over baseline). Thus, high salt diet enhanced the learned stress response but not the startle response. The first conditioned MAP response was greater in CS+ than in CS- trials in both groups. High salt diet enhanced the MAP response in CS+ trials, a finding consistent with that seen for RSNA during the sudden burst. Results similar to this have been reported in SHR: high salt diet caused a greater increase in RSNA (103%) than normal diet (77%) during air jet stress (Koepke et al, 1985).

The relevance of high salt diet to cardiovascular reactivity in humans has been studied by several investigators. For example, Falkner et al (1981) studied reactivity to stress following salt loading in adolescent females with and without a family history of hypertension. Those with a positive family history showed greater stress-induced pressor and bradycardic responses than those with a

negative family history. Also, there are differences in salt sensitivity between hypertensive patients. Kohno and coworkers (1987) reported that one group of hypertensive patients was sensitive to high salt manipulation while another group of hypertensive patients was not. The salt sensitive patients had a greater increase in plasma levels of atrial natriuretic peptide than the non-salt-sensitive patients. As discussed previously, whether the increased plasma atrial natriuretic peptide is a result of hypertension or a factor contributing to chronic hypertension is unknown. Nevertheless, these results were consistent with the present and other animal studies indicating that salt sensitivity may be determined in part by genetics. In other words, genetics leads to salt sensitivity in some people. This sensitivity may increase reactivity to stress, in ways suggesting the involvement of the sympathetic nervous system.

The ratio of RSNA sudden burst change to that for the first conditioned MAP response was 24 for BHR on a normal diet vs. 30 for BHR on high salt diet. The differences between groups were not significant, which suggests that

high salt diet did not change this ratio. It is not clear whether this ratio is strain-specific.

High salt diet also enhanced the RSNA response to CS+ trials during the tone period (sec 15.00-23.99). The increase of RSNA during the tone period in the high salt group was two times higher than that for the normal salt group (17% in BHR-high vs. 6% in BHR) during CS+ trials. The significance level was, however, marginal. This was coupled with a similar bradycardia (-20 bpm) and mild pressor response in both groups. Similar responses in RSNA, HR, and MAP during the quiet period following the sudden burst or conditioned response were found in normal and high salt groups. The inability of high salt to enhance the responses to stress during this tone period was possibly the result of their greater baroreflex gain when compared with normal diet. Actually, BHR on either diet had little increase in RSNA or MAP, combined with a fairly large bradycardia (-30 bpm), during the tone period for CS+ trials. Another compounding factor of reactivity is the duration of the experimental manipulation. Lawler et al (1989) reported that there might be a critical period (age) during which intact

renal nerves are required for the development of stress-induced hypertension in BHR.

Although there were significant differences in HR between trial types during the tone period in each group, differences were not significant between groups or during other periods. The implication is not clear. It seems that high salt diet did not change the tonic level or reactivity of HR in BHR.

The effect of high dietary sodium (8% NaCl) intake was tested only in the BHR strain, for reasons including time limits and costs. We conclude that (1) high salt diet increases baseline MAP, but not HR or RSNA, in BHR animals; (2) high salt diet does not change the general pattern of responses in RSNA, HR, and MAP to classical conditioning; (3) high salt diet enhances the learned responses of RSNA to behavioral stress during the sudden burst and tone periods; (4) high salt diet enhances the conditioned response in MAP, although, the enhanced pressor response appears to be diminished by the baroreflex during the tone period; (5) the unconditioned responses and startle responses in MAP and HR

were not affected differently by dietary sodium intake; and (6) it appears that high salt diet does not change the tonic level or reactivity of HR.

However, the significance of this study has been limited by several weaknesses. First, the effects of high salt diet were studied only in the BHR; second, the role of the parasympathetic nervous system in behavioral stress and its interaction with the sympathetic nervous system in controlling blood pressure and heart rate was not investigated; third, the potential role of the baroreflex was only indirectly assessed; and finally, plasma catecholamine levels were not measured. These are areas all needing further exploration, in order to clarify the role of neural influences on cardiovascular regulation in animals differing in genetics and/or environmental triggers for hypertension.

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APPENDIX

APPENDIX

Composite Files for Each Group during Each Type of Trial

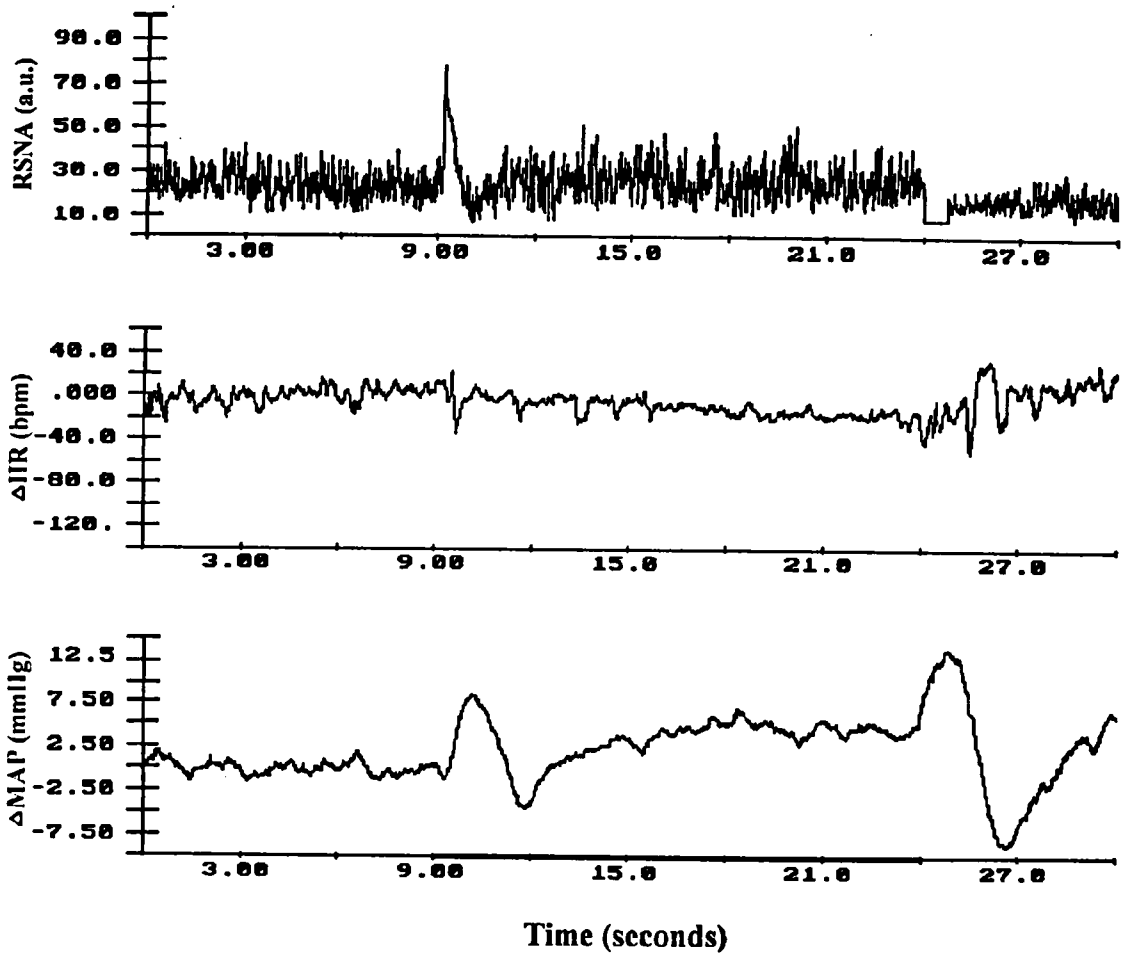

Figure A-1. Composite RSNA, HR and MAP of all WKY during CS+ trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

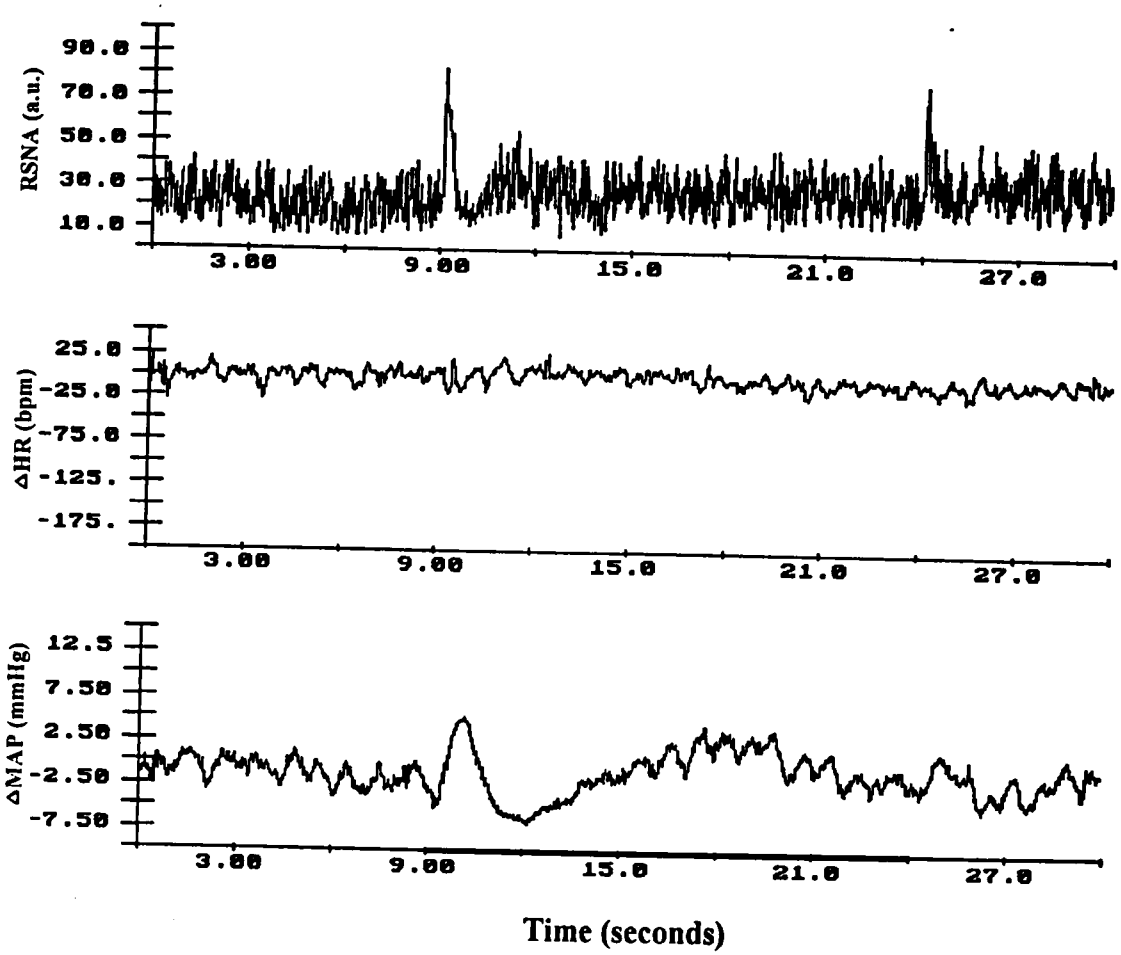

Figure A-2. Composite RSNA, HR and MAP of all WKY during CS- trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

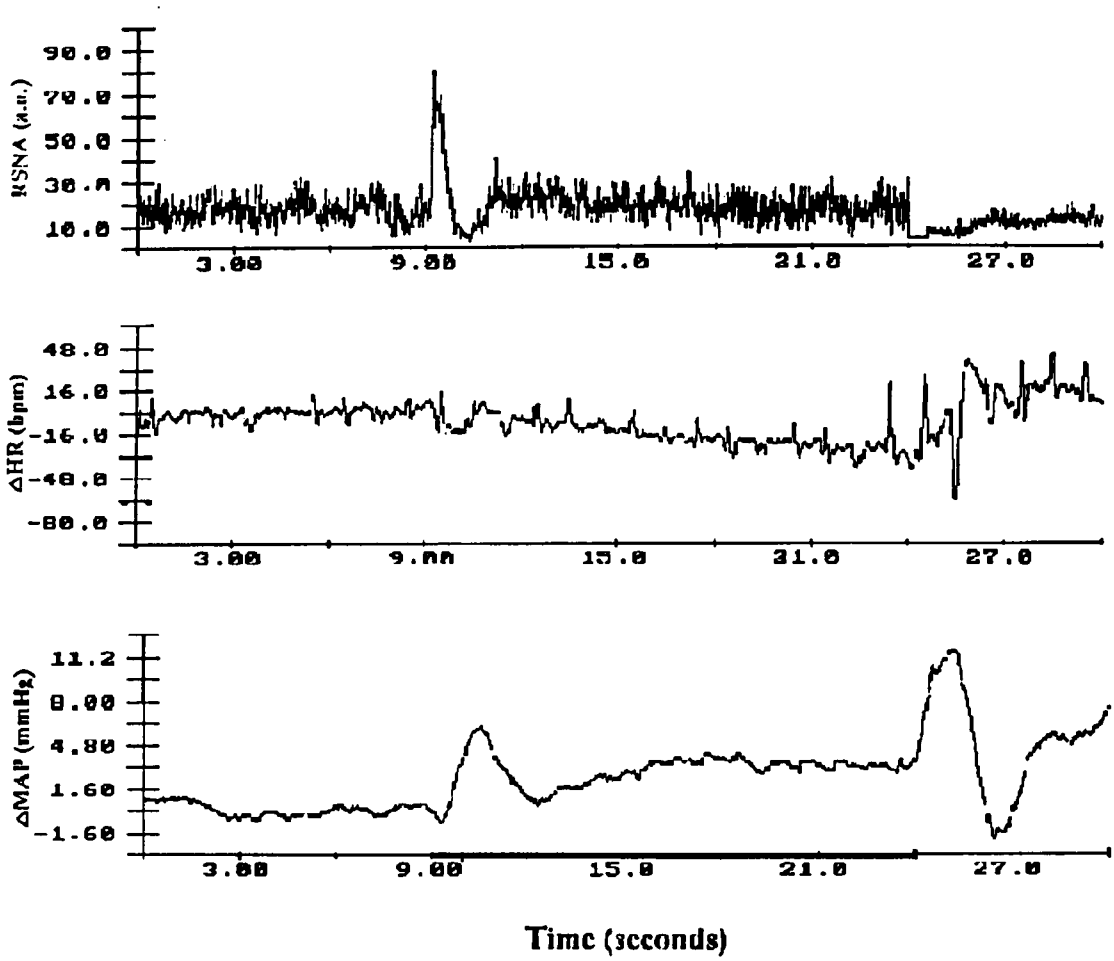

Figure A-3. Composite RSNA, HR and MAP of all BHR on normal diet during CS+ trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

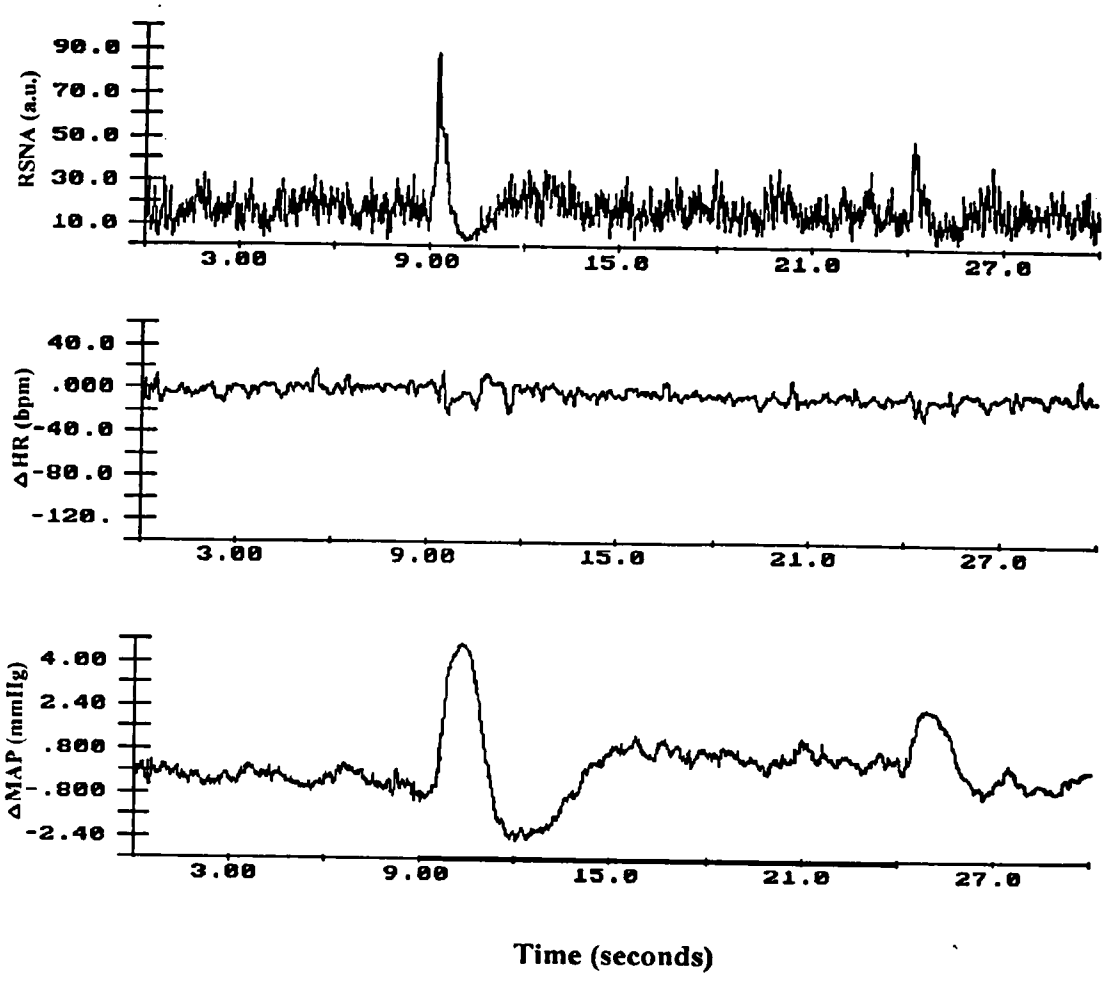

Figure A-4. Composite RSNA, HR and MAP of all BHR on normal diet during CS- trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

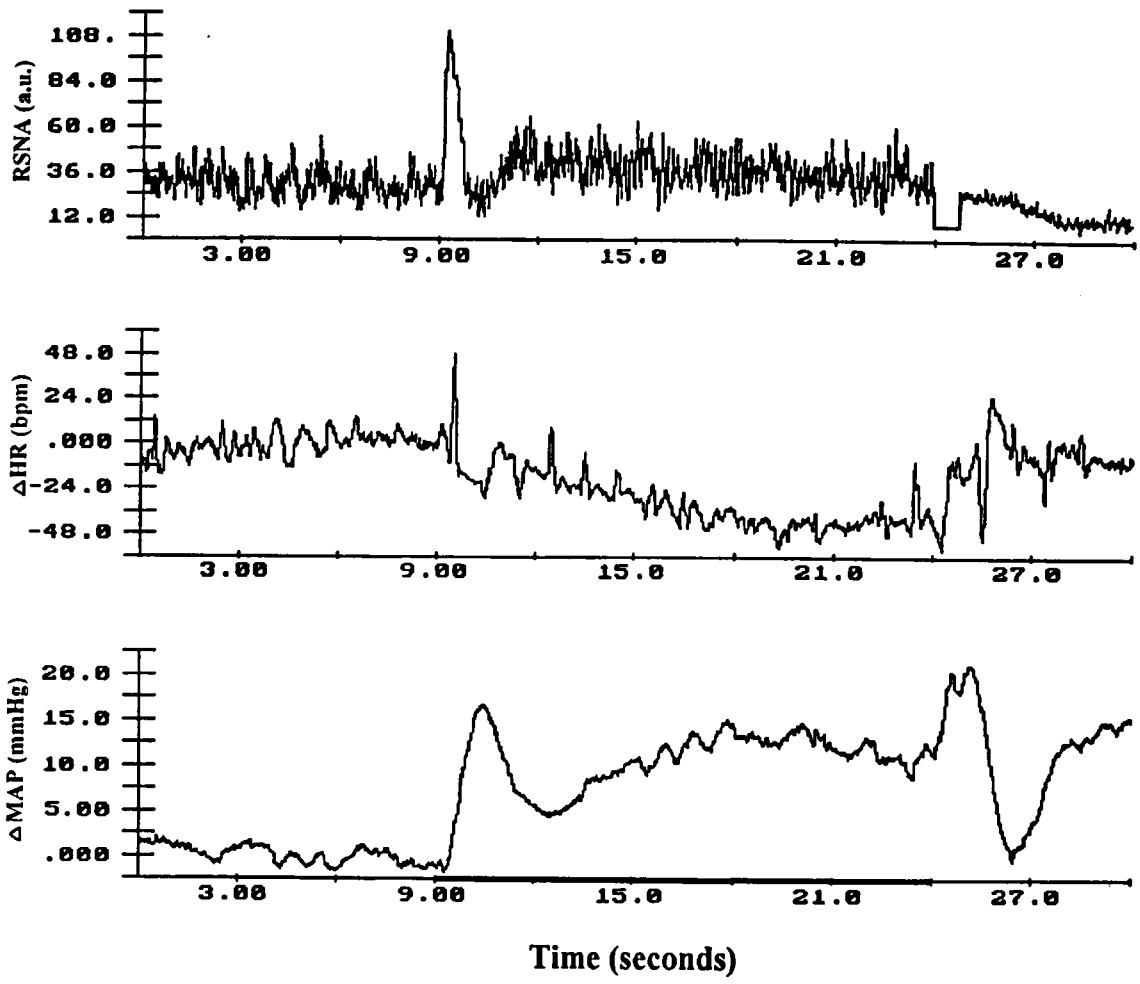

Figure A-5. Composite RSNA, HR and MAP of all SHR during CS+ trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

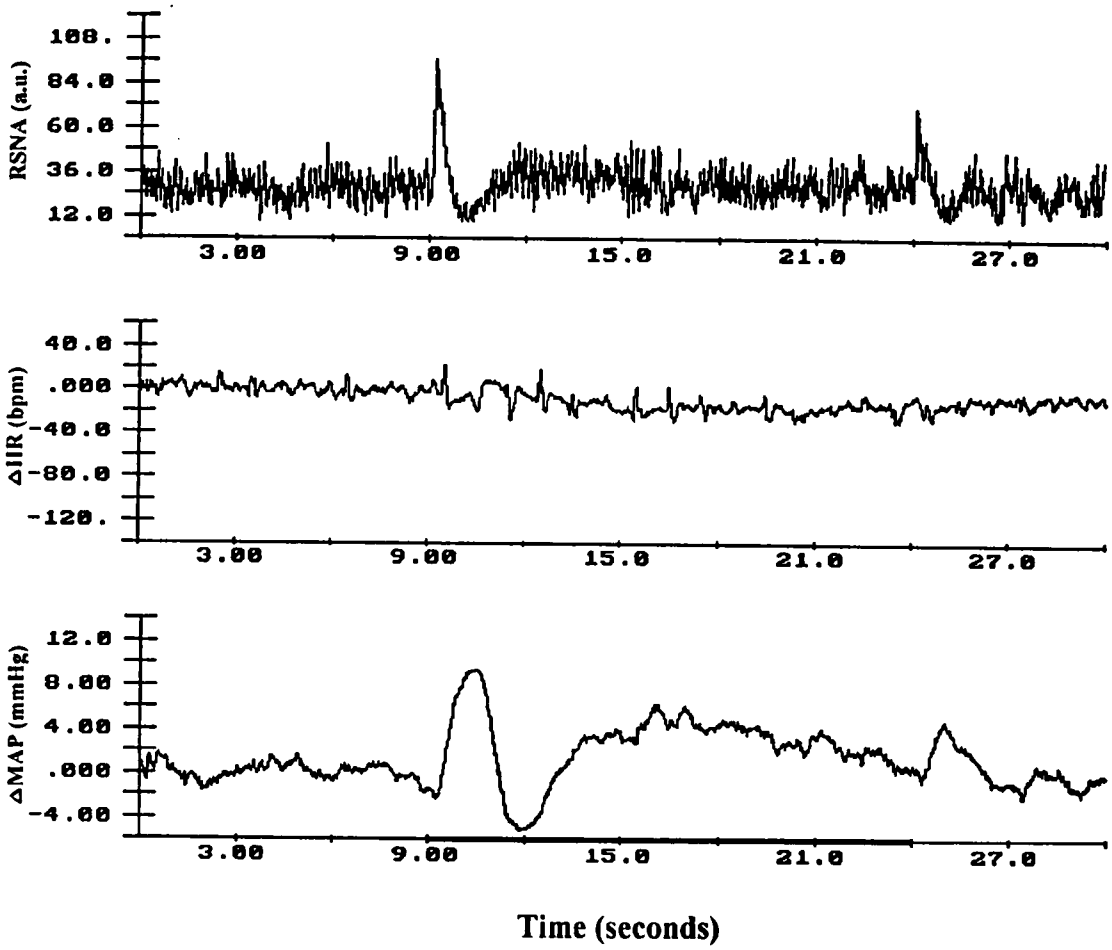

Figure A-6. Composite RSNA, HR and MAP of all SHR during CS-trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

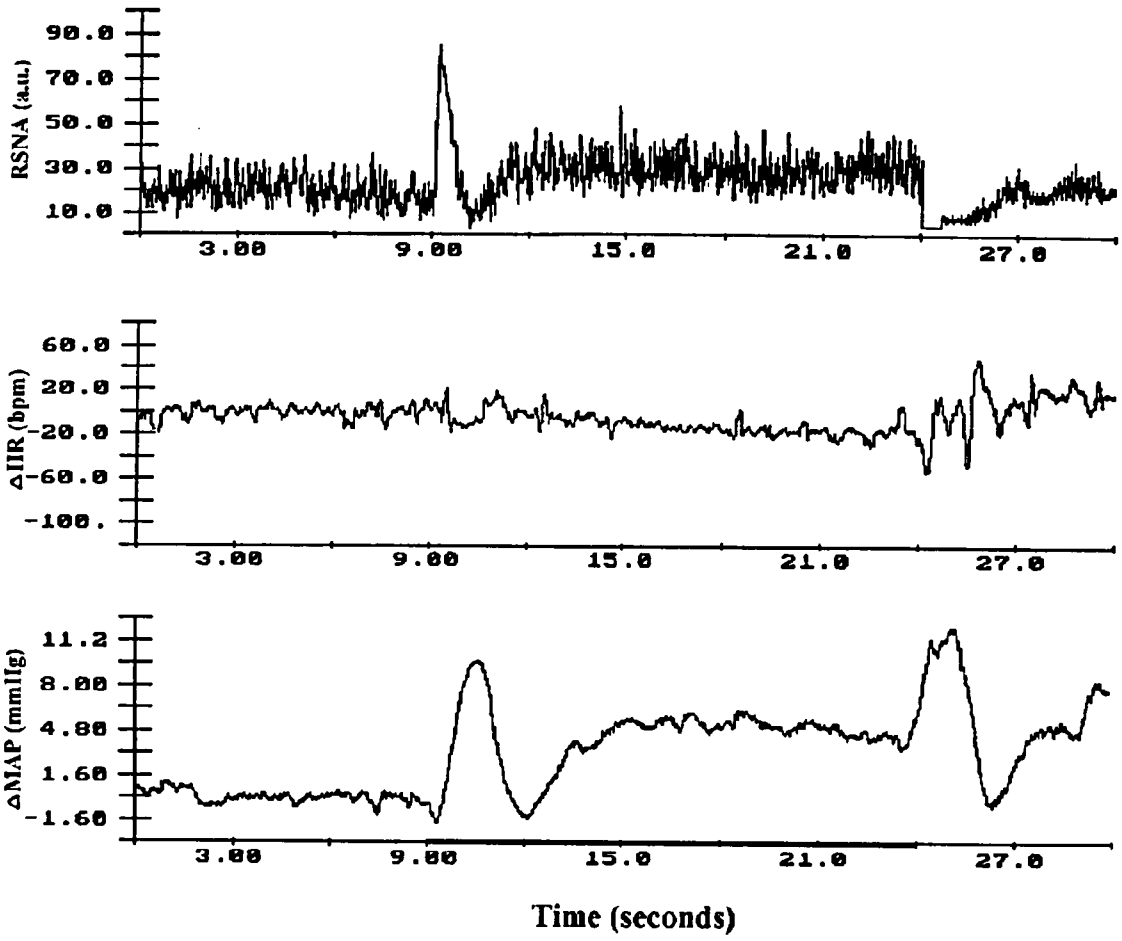

Figure A-7. Composite RSNA, HR and MAP of all BHR on high salt diet during CS+ trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

APPENDIX (continued)

Composite Files for Each Group during Each Type of Trial

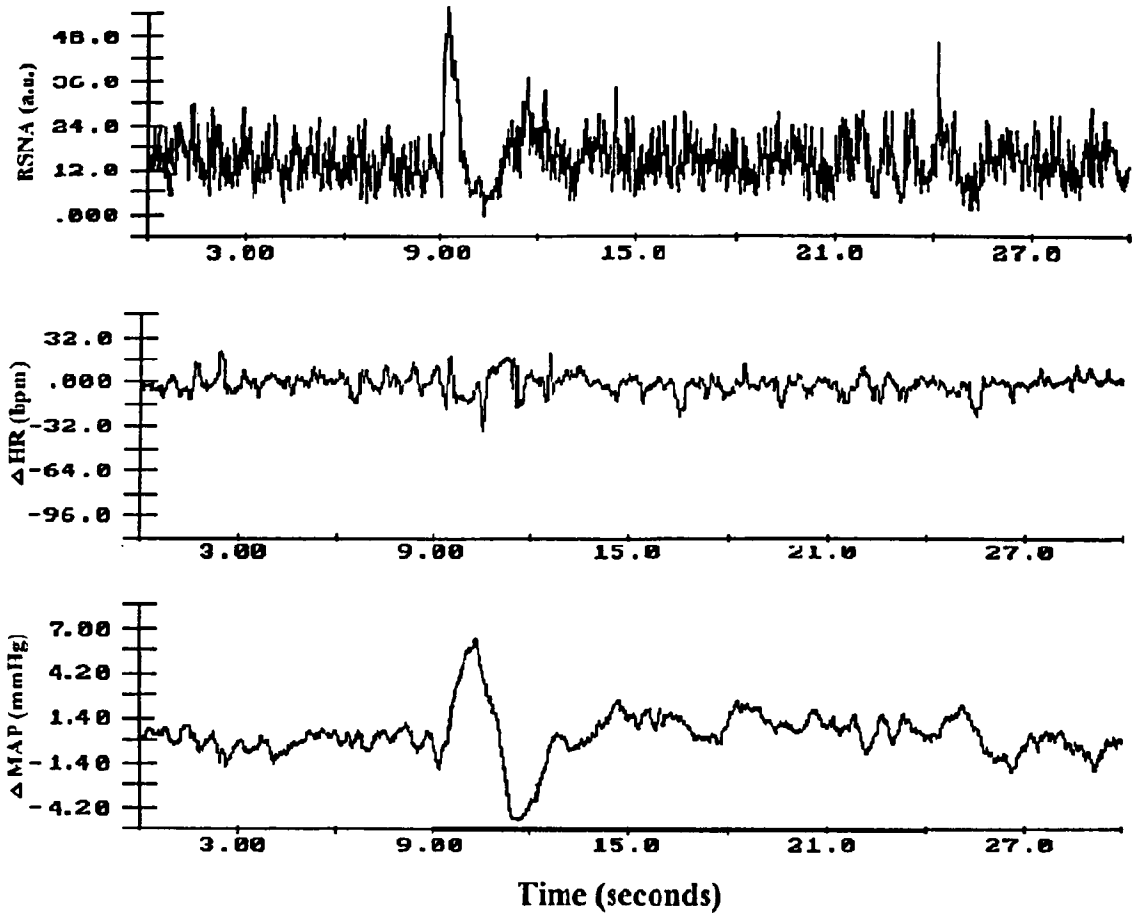

Figure A-8. Composite RSNA, HR and MAP of all BHR on high salt diet during CS- trials. Tone presented during 9.00-23.99 second period (dark bar on time axis).

VITA

Shenggang Li was born in Changde, Hunan Province, People's Republic of China on September 14, 1963. He entered Hunan Medical University during August of 1979 where in August of 1984 he received his MD degree. Then he continued his graduate study in Hunan Medical University in September of 1984 and received a Master degree of Psychiatry and Clinical Psychology in October 1987. After he finished his second degree, he practiced and taught in the Department of Psychiatry, the Second Affiliated Hospital of Hunan Medical University till December 1989. In December 1989, he came to the United States as a visiting scholar in the Department of Psychology University of Tennessee Knoxville. In August of 1991, he entered the University of Tennessee to pursue the Doctorate of Philosophy in Life Sciences, Physiology Program. The doctoral degree was received August, 1995.

Following his graduate training in the University of Tennessee, the author was awarded a postdoctoral fellowship from the American Heart Association Kentucky Affiliate and sponsored by the Department of Physiology University of Kentucky in Lexington, Kentucky.