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Autonomic space analyses of the effects of pharmacological and behavioral alterations of cardiac chronotropic control in the rat

Quigley, Karen Sue, Ph.D.
The Ohio State University, 1993
AUTONOMIC SPACE ANALYSES OF THE EFFECTS OF
PHARMACOLOGICAL AND BEHAVIORAL ALTERATIONS OF CARDIAC
CHRONOTROPIC CONTROL IN THE RAT

Dissertation

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the Degree Doctor of Philosophy in the
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by
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Historically, the study of autonomic nervous control of the viscera can be viewed from two primary perspectives, the reflexive and the psychophysiological. The former perspective is exemplified by studies of autonomic reflexes and adaptive adjustments to homeostatic challenge. In contrast, psychophysiological studies generally have concentrated on the effects of simple (e.g. the orienting reflex) or complex (memory retrieval) psychological manipulations on visceral functions. These perspectives have become increasingly interrelated by current efforts to elucidate the underlying neural and physiological control of target organs during psychological challenges. This literature has revealed that autonomic control may be more flexible and more complex than was traditionally believed. Here, I will emphasize the importance of quantitative approaches in psychophysiology, and outline a recent theoretical model of autonomic control of target organ function and its application to the laboratory rat. A traditional conception of autonomic control of visceral organs entails a unidimensional continuum, ranging from
sympathetic to parasympathetic dominance. Even with this focus on reciprocal coupling of the autonomic branches, investigators such as Gellhorn were already reporting examples of coactivation or independent activation of the autonomic divisions in the mid-20th century (Gellhorn, 1957; Gellhorn, Cortell & Feldman, 1941). Despite the efforts of Gellhorn and others, the idea of a continuum of function from sympathetic to parasympathetic, and the predominance of reciprocal control continues to be reflected in introductory textbooks of physiology and psychology (Gray, 1991; Kandel, Schwartz & Jessell, 1991).

More recent conceptualizations of the function and interrelation of the autonomic branches emphasize the increasing evidence of concurrent or uncorrelated activity in the two branches, as well as the more traditionally observed reciprocal actions of the divisions (e.g. Berntson, Cacioppo & Quigley, 1991; Iwata & LeDoux, 1988; Koizumi, Terui & Kollai, 1983; Koizumi, Terui, Kollai & Brooks, 1982; Quigley & Berntson, 1990). Recent theoretical and empirical conceptions developed in our laboratory maintain that a bivariate model of sympathetic and parasympathetic control is the minimum depiction necessary to capture the complexities of autonomic control of target organ function (Figure 1). This model encompasses the traditional reciprocal modes of autonomic control, as well as the
increasingly recognized coactivational and independent (uncoupled) modes of control of the autonomic branches. In this chapter, I will detail the empirical foundations, general principles, and formal quantitative properties of the autonomic space model. In subsequent chapters, I will instantiate this general autonomic space model for control of cardiac chronotropy in the rat. I will then demonstrate the usefulness of this model for analyzing physiological and psychological responses.
Figure 1. The two dimensional autonomic plane. The autonomic plane is bounded by sympathetic and parasympathetic activation continua expressed as proportional activation units. The diagonal of reciprocity ranges from low sympathetic, high parasympathetic to high sympathetic, low parasympathetic. The diagonal of coactivity extends from low sympathetic, low parasympathetic to high sympathetic, high parasympathetic. Uncoupled autonomic modes of control are depicted along the marginal axes and along parallels to the axes.
Figure 1.
Basic Principles of Autonomic Space

The unidimensional, reciprocal model

The traditional unidimensional conception of the autonomic nervous system encompasses three, implicit assumptions about autonomic control of target organs: (a) visceral target organs are dually innervated by sympathetic and parasympathetic autonomic nerves, (b) sympathetic and parasympathetic innervations to a target organ are functionally antagonistic, and (c) target organs are under a reciprocal mode of central control (Berntson, Cacioppo & Quigley, 1991). These assumptions do not incorporate the accumulated evidence for singly innervated target organs, synergistic sympathetic and parasympathetic actions on a given target organ, or the numerous occurrences of non-reciprocal actions of the sympathetic and parasympathetic divisions.

The autonomic space model

The broader model of autonomic space takes as its starting point the two-dimensional bivariate autonomic plane bounded by the full range of activity of both sympathetic and parasympathetic divisions (Figure 1; Berntson, Cacioppo, & Quigley, 1991). The autonomic space model permits reformulation of the implicit assumptions of the traditional model as follows: (a) visceral target organs may be
innervated by one or both autonomic divisions, (b) autonomic inputs to a dually innervated target organ may exhibit antagonistic or synergistic functional effects on that organ, and (c) the control of sympathetic and parasympathetic outflows to a target organ may be inversely correlated, directly correlated, or uncorrelated. This broader model subsumes evidence that not all target organs are dually innervated, and that autonomic innervations may act synergistically to affect target organ function (Fowles, 1986; Loewy & Spyer, 1990).

The primary interest here is the recognition that there are three basic classes of relationships between the autonomic branches: (a) a reciprocal mode when the relationship between the activity of the branches is negatively correlated, (b) a non-reciprocal (coactive or coinhibitive) mode when activities of the branches are positively correlated, and (c) an uncoupled mode when the relationship between the activity of the branches is uncorrelated. These modes are represented by the vectors on the autonomic plane depicted in Figure 1. The reciprocal modes lie along vectors parallel to the reciprocal diagonal of this plane (from low sympathetic, high parasympathetic to high sympathetic, low parasympathetic). Non-reciprocal modes lie along vectors parallel to the coactivity diagonal (from high sympathetic, high parasympathetic to low sympathetic, low parasympathetic). Finally, the uncoupled
modes lie along vectors parallel to the marginal axes of the autonomic plane. These three classes of modes (and time-varying responses which may include combinations of these modes) comprise an exhaustive set of the possible relationships between the autonomic branches.

Empirical documentation of each of these modes can be found in the literature (see Berntson, Cacioppo & Quigley (1991) for a comprehensive review). Reciprocal modes of control have long been documented for such important reflexive functions as the baroreceptor-heart rate reflex (Head & McCarty, 1987). Cohen and Pitts (1968) also demonstrated a reciprocal mode of control underlying a classically conditioned heart rate acceleration in pigeons. Coupled modes of coactivation or coinhibition have also been demonstrated in both physiological and psychological contexts. By direct recording of activity in cardiac nerves, Kollai & Koizumi (1979) demonstrated coactivation of sympathetic and parasympathetic control of the heart during progressive hypoxia in the dog. Using pharmacologic autonomic blockade, Iwata & LeDoux (1988) demonstrated that although conditioned and pseudoconditioned rats exhibited virtually identical heart rate responses to a conditioned stimulus, the mode of control underlying the response in pseudoconditioned animals was primarily an uncoupled sympathetic activation, whereas the conditioned animals exhibited a pronounced coactivation of both sympathetic and
parasympathetic controls. Finally, uncoupled modes of autonomic control have also been demonstrated in both physiological and psychological paradigms. Although exercise ultimately results in a reciprocal mode of autonomic action on the heart, the predominant initial effect is a progressive withdrawal of parasympathetic activity with little alteration in sympathetic outflows to the heart (Robinson, Epstein, Beiser & Braunwald, 1966; Rowell, 1986). Additionally, Quigley & Berntson (1990) demonstrated a cardioacceleratory response to a defensive auditory stimulus that was predominantly mediated by sympathetic activation, with little contribution from the parasympathetic division. This brief sample suggests a wide variety of conditions under which each of the possible modes of autonomic control may obtain. In the face of these multiple modes of control, it is clear that a bivariate model is necessary to encompass the complexities of autonomic control.

Quantitative Model of Autonomic Space

The model of autonomic space can be characterized in mathematical form such that the functional state of the target organ is expressed as a function of independent sympathetic and parasympathetic activity. The autonomic space model outlined below is an adaptation of the models of Levy and Zieske (1969), and Warner and Cox (1962) for the
autonomic control of the heart. These models were based on
direct stimulation of the autonomic nerves, however, and
describe the potential relationships between autonomic
activities and the chronotropic state of the heart. In
contrast, the autonomic space model outlined here focusses
on the normal control of autonomic outflows, and on the
functional states arising from different modes of control.
The sympathetic and parasympathetic activation functions
form the x and y axes of the autonomic plane, respectively,
and the functional state of the target organ can be depicted
perpendicular to this plane along the z axis for any x, y
coordinate point. The general form of the quantitative
autonomic space model is as follows:

\[(1) \quad f_{ij} = B + C_s * S_i + C_p * P_j + I_{ij} + \epsilon,\]

where \(f_{ij}\) is the functional state of the target organ for
any point \(i, j\) on the sympathetic and parasympathetic
activation continua, respectively, \(B\) is the intrinsic
functional state of the target organ in the absence of
autonomic control, \(S_i\) and \(P_j\) are the independent functional
sympathetic and parasympathetic activations of the target
organ for the point \(i, j\), \(C_s\) and \(C_p\) are the coupling
coefficients that capture the relative functional effect of
sympathetic and parasympathetic activities on the target
organ at point \(i, j\), \(I_{ij}\) is a term capturing potential
interactions of the sympathetic and parasympathetic
divisions, and $\epsilon$ is an error term that includes both
measurement error and systematic error from non-autonomic
sources such as hormonal or metabolic effects on the target
organ.

Although equation 1 represents only a generalized model
of autonomic control, derivation of the parameters of this
equation for a given organism permits the development of a
specific implementation for that organism. In subsequent
chapters, I will develop this quantitative model for
chronotropic control in the rat. I will then illustrate the
importance of quantitative approaches to the study of
autonomic control, and the utility of the autonomic space
model for psychophysiological studies.
CHAPTER II

INSTANTIATION OF THE AUTONOMIC SPACE
MODEL IN THE RAT

The autonomic space model provides a general conceptual representation of autonomic control and consequence, and equation 1 allows for a particular instantiation of autonomic control for a given target organ and species. Because rats are increasingly being used to study a variety of cardiovascular phenomena, a relatively complete literature exists on the features needed to model autonomic space in the rat. In addition, because rats are easily handled and instrumented, and display a wide range of well-documented somatic and cardiovascular behaviors, they serve as appropriate subjects in studies of simple emotional, attentional, motivational or other cognitive behaviors. I will begin by enumerating the features of the autonomic space equation needed to instantiate autonomic space for the chronotropic control of the heart. I will then review several reports in the literature, and present a study from our laboratory which together provide the necessary parameters for an estimated autonomic space in the Sprague-Dawley rat (Berntson, Cacioppo, Quigley & Fabro, in press).
The autonomic space equation (equation 1) includes terms for the intrinsic chronotropic state of the heart, the independent sympathetic and parasympathetic contributions to the chronotropic function of the heart, the potential interactions between the sympathetic and parasympathetic branches, and an error term encompassing both measurement and systematic error from non-autonomic sources.

\[ f_{ij} = \beta + C_s \cdot S_i + C_p \cdot P_j + I_{ij} + \epsilon \]

The following terms define the relevant parameters necessary for a specific instantiation of the autonomic space model: (a) the chronotropic function of the heart in the absence of neural control (intrinsic heart period or \( \beta \)), (b) the dynamic ranges of sympathetic and parasympathetic chronotropic control which define the relative contributions of each division, and determine values for the weighting coefficients, \( C_s \) and \( C_p \), and (c) an estimate of the nature and extent of potential sympathetic/parasympathetic interactions, or an estimation of \( I_{ij} \).

\[ ^1 \text{The coefficients } C_s \text{ and } C_p \text{ are represented here as constants. Although they could vary as a function of the absolute levels of } S_i \text{ and } P_j, \text{ we will show subsequently that this is not the case for the instantiation here.} \]
**Intrinsic heart period (β)**

The intrinsic chronotropic state of the heart can be indexed by either combined sympathetic and parasympathetic autonomic blockade, by cardiac autonomic denervation, or by ganglionic blockade. Although cardiac denervation may provide an estimate of intrinsic chronotropic state of the heart, long term alterations such as re-innervation, or receptor up- or down-regulation, can occur in the denervated heart. Although these long-term readjustments are minimized in the acute preparation (e.g. Billman, Hoskins, Randall, Randall, Hamlin, and Lin, 1989; Randall, Kay, Randall, Brady & Martin, 1976; Randall, Thomas, Barber, & Rinkema, 1983), a recent study by Randall, Randall, Brown, Yingling and Raisch (1992) suggested that denervations may disrupt important trophic influences of the autonomic innervations, even in the acute condition. Likewise, ganglionic blockade is typically less useful than more specific autonomic blockade agents because relatively selective blockades have minimal effects on other organ systems. When indexed by combined blockade of sympathetic and parasympathetic effector synapses, relatively similar estimates of intrinsic heart period for the Sprague-Dawley rat emerge across laboratories and studies. Head & McCarty (1987) report dual blocked mean heart periods of 158 msec, Corre, Cho & Barnard (1976), 161 msec, and Berntson, Cacioppo, Quigley & Fabro (in press), 166 msec. The mean of these values, 162 msec can serve as
an estimate of the intrinsic chronotropic state of the heart in the Sprague-Dawley rat. This value also compares well with the combined effects of single blockades in our laboratory (160 msec; using the method of Lin & Horvath, 1972) as well as with estimates from other rat strains (Wistar = 166 msec, Lin, 1974; Wistar-Kyoto = 169 msec, Murphy, Sloan & Myers, 1991).

Dynamic range (coupling coefficients)

The relative functional impact of the autonomic branches are represented by the values of the coefficients $C_s$ and $C_p$. With $S_i$ and $P_j$ expressed in proportional units of activation (from 0 to 1), $C_s$ and $C_p$ represent the dynamic ranges (in msec of heart period) for the sympathetic and parasympathetic divisions, respectively. Note that $B$ serves as the zero activation level for both autonomic divisions, and thus the dynamic ranges for each division are given by the following equations (2 & 3):

\begin{align*}
(2) \quad C_s &= HPS_{\text{max}} - B \\
\text{and} \\
(3) \quad C_p &= HPP_{\text{max}} - B
\end{align*}

where $HPS_{\text{max}}$ and $HPP_{\text{max}}$ are the heart periods under maximal sympathetic and parasympathetic control,
respectively. Thus, the coupling coefficients are derived from an estimate of $\beta$ and estimates of heart period limits under maximal sympathetic and parasympathetic control.

**Maximal sympathetic control.** An estimate of maximal sympathetic control of heart period can be derived from three sources: maximal exercise, chronotropic effects of adrenergic agonists, and baroreflexive sympathetic drive. In the rat at maximal exercise, virtually complete parasympathetic withdrawal occurs, as indicated by the lack of effect of cholinergic antagonists on heart period (Corre et al., 1976; Ekblom, Goldbarg, Kilbom, & Astrand, 1972). Moreover, sympathetic activation is relatively complete as evidenced by the failure of the $\beta$-adrenergic agonist, isoproterenol to further shorten heart period (Bolter & Atkinson, 1988a). Heart period during maximal exercise in the rat, however, is also affected by thermal effects on the sinoatrial node. When thermal effects are removed (Bolter & Atkinson, 1988b), maximal sympathetically-mediated exercise heart period is estimated at 120 msec in the Sprague-Dawley rat (Bolter & Atkinson, 1988b; Corre et al., 1976; Sonne & Galbo, 1980). The $\beta$-adrenergic agonist isoproterenol results in highly similar minimal heart periods of 121 msec, and 122 msec, *in vitro* and *in vivo*, respectively (Bolter & Atkinson, 1988a). Finally, nitroprusside infusions that result in maximal baroreflexive drive on sympathetic outflow
to the heart yield a minimum heart period of 119 msec. These three estimates of maximal sympathetic control are highly consistent (119-122 msec) and yield an overall estimate of $H_{P_{max}} \approx 120$ msec.

**Maximal parasympathetic control.** Estimates of maximal parasympathetic control in the rat can be derived from potent parasympathetic reflexes such as the dive reflex, and from direct stimulation of parasympathetic efferent fibers to the heart. The dive reflex, evoked by facial submersion and mediated by trigeminal and chemoreceptor afferents, entails a massive increase in vagal outflow to the heart coupled with a virtually complete sympathetic withdrawal (Lin, 1974). Its predominant vagal mediation is suggested by the elimination of diving bradycardia by vagal blockade, and by the lack of effect of either sympathetic blockade or adrenergic depletion by reserpine (Lin, 1974). Studies of facial immersion in unanesthetized rat revealed maximal heart periods of 403 msec (Huang & Peng, 1976) and 419 msec (Lin, 1974) which provide an estimate of $H_{P_{pmax}} \approx 411$ msec. Converging evidence for this estimate, comes from vagal stimulation (to be reported in the next section), which yields a maximal heart period of 401 msec. A reasonable value for maximal parasympathetic control is the mean of these highly consistent estimates, or $H_{P_{pmax}} \approx 406$ msec.
Sympathetic and parasympathetic transfer functions

The literature reveals that cardiac chronotropy expressed in heart period, rather than heart rate, exhibits a linear relationship with sympathetic and parasympathetic outflows. Figure 2 depicts the results of several studies of direct stimulation of autonomic nerves in mammals. The data of Figure 2 demonstrate the essential linearity of the relationship between the frequency of cardiac efferent activity and cardiac chronotropy expressed as heart period. This relationship has been confirmed for humans, dogs, cats and rabbits (Carlson, 1992; Carlsten, Folkow & Hamberger, 1957; Ford & McWilliam, 1986; Furnival, Linden & Snow, 1973; Furukawa, Wallick, Carlson & Martin, 1990; Masuda & Levy, 1985; Neely & Urthaler, 1992; Parker, Celler, Potter & McCloskey, 1984; Rosenblueth & Simeone, 1934; Stramba-Badiale, Vanoli, DeFerrari, Cerati, Foreman, & Schwartz, 1991; Versprille & Wise, 1971). Direct recording studies reveal that spontaneous fluctuations in cardiac efferent activity also display an essentially linear relationship with heart period (Jewett, 1964; Katona, Poitras, Barnett, & Terry, 1970; Koizumi, Terui, & Kollai, 1985).
Figure 2. Heart period effects of parasympathetic and sympathetic efferent stimulation. The left panel illustrates the essentially linear relationship between vagal efferent stimulation frequency and heart period for the human (Carlsten, Folkow & Hamberger, 1957), dog (Parker, Celler, Potter & McCloskey, 1984) and rabbit (Ford & McWilliam, 1986). Similar data are shown for the rat in Figure 4 (Berntson, Quigley, Fabro & Cacioppo, 1992). The right panel illustrates the essentially linear relationship between sympathetic efferent stimulation and heart period for the dog (Furnival, Linden & Snow, 1973; Masuda & Levy, 1985; Neely & Urthaluer, 1992).
Cardiac Vagal Efferent Stimulations
Human, Dog and Rabbit (all N = 1)

Heart Period (msec)

Stimulation Frequency (Hz)

Cardiac Sympathetic Efferent Stimulations
Dog

Heart Period (msec)

Stimulation Frequency (Hz)

Figure 2.
Although linearity may seem surprising in a physiological system, the quantitative model of Dexter, Levy and Rudy (1989) reveals that the linearity in the parasympathetic outflows arises from two non-linear processes. This model notes that acetylcholine (ACh) accumulation at cardiac synapses is a negatively accelerating function of vagal efferent firing frequency, and that cardiac chronotropy is a positively accelerating function of ACh concentration at the cardiac synapses. These two non-linear functions yield the resultant linear relationship between the frequency of cardiac efferent activity and cardiac chronotropy.

The linearity between autonomic outflows and cardiac chronotropy is important because a unit change in autonomic outflow yields a constant heart period change regardless of the basal heart period level. Because data on the transfer function between parasympathetic efferent outflows and heart period did not exist for the rat, we conducted direct stimulation studies of the cardiac vagal efferents.

**Vagal stimulation in the rat**

**Subjects and experimental apparatus.** Subjects were 5 male Sprague-Dawley rats (250-450 g; Zivic Miller, Zelienople, PA). Subjects were anesthetized with urethane (1.4 g/kg) and supplemented as necessary. The left common carotid was cannulated (Vascular Access Port, Model SLA,
Norfolk Medical Products, Skokie, IL), the left vagus nerve was transected, and the right vagus was isolated by careful dissection. The catheter was coupled to a Spectramed TNF-R pressure transducer (Spectramed, Oxnard, CA) and then to a Grass Model 7 polygraph (Grass Instruments, Quincy, MA). The ECG was recorded via subcutaneous electrodes, and both the pressure wave and the ECG were recorded on chart paper (30 mm/sec). For time-critical measures, the signals were digitized (Metrabyte DAS-8, 500 Hz, 12 bit) and stored for offline analysis.

Prior to stimulation, the cardioselective β-adrenergic antagonist, atenolol was administered (10 mg/kg, s.c.) to minimize confounding from potential basal or reflexive sympathetic activation. The distal end of the transected right vagus was placed over a stainless steel hook electrode (0.2 mm diameter), and the stimulation field was drenched with mineral oil to prevent drying and minimize current spread. Monophasic cathodal pulses from a Grass Model S6 stimulator (Grass Instruments) were delivered by the stimulation electrode which was referenced to a remote indifferent. Ascending and descending series of discrete stimulation frequencies were utilized to characterize optimal stimulus parameters, and to derive the frequency vs. heart period relationship. For analysis, mean heart period and blood pressure were determined from 10-15 sec after
stimulation onset, and from the 5 sec period immediately preceding stimulation.
Results. Figure 3 illustrates the stimulus parameters and basal heart period values following drug administrations and nerve transection. Atenolol yielded the typical increase in heart period associated with sympathetic blockade, and bilateral vagotomy decreased basal heart period consistent with a tonic parasympathetic contribution to heart period (Figure 3, panel a). To test the completeness of sympathetic blockade, the vasodilator nitroprusside was administered (5 mg/kg, s.c.) to stimulate baroreflexive sympathetic drive. Sympathetic blockade was confirmed when nitroprusside resulted in virtually no change in heart period, despite a notable decrease in mean blood pressure (mean = -45.5 mmHg). Voltage (5 V) and pulse duration (0.2 msec) parameters were chosen to maximally drive the vagal nerve on the basis of asymptotic response in preliminary studies (Figure 3, panels c & d). The temporal characteristics of the response to stimulation are depicted in Figure 3 (panel b). To characterize the shape of the transfer function of the vagal cardiac efferents, a wide range of stimulation frequencies were employed, from below threshold (0.2 Hz) to levels resulting in sinus block or other arrhythmias (maximum = 30 Hz). For three subjects, two separate stimulation series were conducted to examine the replicability of the response and to confirm integrity of the nerve. These replications are depicted in the upper right and lower panels of Figure 4.
Figure 3. Vagal stimulation parameters and manipulation checks. (a) Basal heart period after experimental manipulations (means ± SEM, n = 3). Bsl: Mean heart period under urethane anesthesia and after left vagal transection. Atenolol: Mean heart period in the same animals after subsequent administration of atenolol (10 mg/kg). Vagot: Mean heart period after section of the right vagal nerve. Baro: Heart period change after nitroprusside-induced hypotension. (b) Time course of heart period change with stimulation of the right vagal nerve. (c) Heart period change during vagal stimulation as a function of stimulus pulse width (5 volts at 10 Hz). (d) Heart period change during vagal stimulation as a function of stimulation voltage (0.2 ms pulse width at 10 Hz).
Figure 4. Effects of vagal stimulation frequency on heart period in the rat. **Upper left panel:** Individual (dashed) and overall (solid) regression functions relating heart period to vagal stimulation frequency. **Other panels:** Illustrative data from 3 subjects from which replicate stimulation series were obtained. Dashed lines depict results from two separate stimulation series, and the solid line illustrates the linear best fit. Stimulation consisted of 0.2 ms monophasic cathodal pulses at 5 volts.
Figure 4.

Simulation Frequency (Hz)

Heart Period (ms)

Equation 1:
\[ y = 1.35x + 17.5 \]

Equation 2:
\[ y = 5.53x + 22.8 \]

Equation 3:
\[ y = 6.89x + 12.73 \]

Equation 4:
\[ y = 7.39x + 19.68 \]
Reference to Figure 4 indicates that increasing frequencies of vagal stimulation resulted in progressive increases in heart period. Despite some deviations within individual subjects, the relationship between stimulation frequency and heart period was closely approximated by linear functions in all subjects (below the level producing sinus block or other arrhythmias). The regression R²'s for each subject revealed that linear trends accounted for 93-99% of the variance in heart period (mean ± SD = 0.97 ± 0.02).

Although the regression slopes differed somewhat across the 5 subjects (Figure 4), the overall linear regression function (slope = 7.4 ms/Hz) still accounted for approximately 80% of the variance in heart period across subjects (F = 176.5, df = 1, 45, p < 0.01). Moreover, despite differences in baseline heart period, there was a striking consistency across animals in the heart period at which heart block or other arrhythmias became apparent (390-420 msec, mean = 401 msec). This closely approximates the HPP\textsubscript{max} estimate derived above from the dive reflex in the rat (411 msec). Although increasing stimulation frequency could result in longer heart periods (up to 860 msec), these periods are outside of the range of normal physiologic function, and generally were indicative of arrhythmias.

Discussion. These results are in accord with numerous studies demonstrating a relatively linear relationship
between vagal outflow and heart period in mammalian species (see above). The present results also indicate a maximal, non-arrhythmic heart period of approximately 401 msec, which converges with the $HPP_{\text{max}}$ estimate derived from the dive reflex (411 msec).

From the literature reviewed and the results of vagal stimulation reported here, it appears that a linear model generally captures the transfer function relating sympathetic and parasympathetic activity and cardiac chronotropic state when expressed as heart period across a wide range of species. Therefore, a linear function will be used to approximate the shape of the autonomic surface for the Sprague-Dawley rat.

**The quantitative instantiation of the rat autonomic space model**

The essentially linear relationship between heart period and autonomic outflows permits a specification of the coefficients $C_s$ and $C_p$ as constants, rather than vectors. The estimates of maximal sympathetic and parasympathetic control derived above, along with the estimated intrinsic heart period ($\beta$) can now define $C_s$ and $C_p$ using equations 2 and 3 as follows:

\[
(4) \quad C_s = 120 - 162 = -42
\]

and

\[
(5) \quad C_p = 406 - 162 = 244
\]
The autonomic space equation in the absence of an interaction term becomes:

\[
(6) \quad f_{ij} = 162 - 42 \cdot s_i + 244 \cdot p_j + I_{ij} + \epsilon
\]

Equation 6 yields a cardiac chronotropic effector surface which represents the possible chronotropic states for all loci within autonomic space. The rat autonomic space is illustrated in Figure 5, with the marginal axes represented in proportional activation units ranging from 0 to 1 (\(S_i\) and \(P_j\)). For illustration, the autonomic axes are scaled in proportion to their relative dynamic ranges (i.e. by the magnitude of \(C_s\) and \(C_p\)). For such a depiction, heart period changes are equivalent for displacements of a given distance across the autonomic surface regardless of the direction in which movement occurs. This depiction also illustrates the greater range of control over chronotropic function of the parasympathetic division relative to the sympathetic division (approx. 6:1). Indeed, the broader range of parasympathetic chronotropic control appears to be a general feature of mammals (Berntson, Cacioppo & Quigley, in press; Mace & Levy, 1983a, b; Neely & Urthaler, 1992; Warner & Russell, 1969).

Autonomic space depictions represent the translation of autonomic outflows to chronotropic function. This translation is distinct from the function relating central
states to autonomic outflows. The linearity of the transfer function between autonomic outflows and heart period does not imply that translations of central state to autonomic outflow will also be linear. Indeed, baroreflex-mediated alterations in heart period approximate a sigmoidal function with respect to increments in blood pressure indicative of a non-linear central mechanism. Although, autonomic space depictions represent only one translational stage in the central mediation of heart period, surface maps may also be used to depict the relationship between central state and autonomic outflow once the transfer function is defined.
Figure 5. Rat autonomic space. The two dimensional autonomic plane and overlying functional surface are depicted for the rat as derived from equation 6. The marginal axes are represented in proportional units such that a unit of movement in any direction within the autonomic plane results in a unit of change in heart period. Dotted lines on the autonomic plane represent isofunctional contours projected down from the overlying functional surface, and illustrate loci in autonomic space having equivalent chronotropic effects.
Interactions

Numerous interactions between the sympathetic and parasympathetic innervations of the heart have been observed at both pre- and post-junctional sites (Levy, 1990). A pre-junctional inhibitory influence of acetylcholine (ACh) on sympathetic, adrenergic terminals has been well established (Loffelholz & Muscholl, 1969, 1970). A similar mechanism has been proposed to account for inhibition of ACh by norepinephrine (NE) at parasympathetic terminals (Levy, 1990; but see Manabe, Foldes, Torocsik, Nagashima, Goldiner, and Vizi, 1991). In addition, neuropeptide Y (NPY), known to be colocalized and released with NE at sympathetic terminals, has also been implicated in exerting a relatively longer term inhibitory effect on ACh release from parasympathetic terminals (Potter, 1987; Warner & Levy, 1989a, b, 1990). Finally, a post-synaptic adenylate cyclase-dependent mechanism may also contribute to sympathetic-parasympathetic interactions (Isenberg & Belardinelli, 1984; Lerman, Wesley, DiMarco, Haines, & Belardinelli, 1988; Levy, 1990). Together, these results suggest that cardiac autonomic interactions are complex.

The most commonly observed chronotropic interaction is a parasympathetic inhibition of sympathetic effects on the heart, termed accentuated antagonism (Furukawa & Levy, 1984; Henning, Khalil & Levy, 1990; Levy & Zieske, 1969; Stramba-Badiale, Vanoli, DeFerrari, Cerati, Foreman & Schwartz,
1991; Urthaler, Neely, Hageman, & Smith, 1986). For a given level of parasympathetic activation, accentuated antagonism of cardiac chronotropy manifests as a progressively slower heart rate with increasing sympathetic activation (Stramba-Badiale, et al., 1991, Figure 3; Urthaler et al., 1986).

Studies on sympathetic and parasympathetic interactions have typically employed heart rate as the metric of cardiac chronotropism. This is potentially problematic, as heart rate is a non-linear transform of heart period. Whereas heart period demonstrates an essentially linear relationship with autonomic outflows, the same is not true of heart rate. Indeed, substantial increases in heart rate induced by sympathetic stimulation can alter the scaling of changes in autonomic outflow arising from different tonic heart rate levels. These considerations suggest that heart period may be a more mechanistically and conceptually appropriate metric for determining the effects of autonomic interactions on cardiac chronotropic control (Berntson, Cacioppo, Quigley & Fabro, in press; Parker, et al., 1984).

A classic interaction study is that of Levy and Zieske (1969) who used direct sympathetic and parasympathetic stimulation to estimate autonomic interactions in dogs. Using the reported basal heart rate of 180 bpm, and the equation containing the derived polynomial interaction term, the Levy and Zieske (1969) results are illustrated in the left panel of Figure 6. An interaction suggestive of
accentuated antagonism is apparent over the range of parasympathetic stimulation, as parasympathetic effects are progressively enhanced at higher levels of sympathetic stimulation. The heart rate values were converted to heart period and are depicted in the right panel of Figure 6. The converted heart period data are distinctly linear with the exception of the highest frequencies of vagal stimulation. Moreover, the deviation in heart period as a result of different levels of sympathetic stimulation cannot represent an interaction, as this deviation is most apparent where sympathetic stimulation is null and no interaction can occur. This result instead suggests that the highest levels of vagal stimulation used by Levy and Zieske (1969) reach asymptotic levels of chronotropic control. These data emphasize the importance of the chronotropic metric in estimating autonomic interactions. Most or all of the apparent autonomic interaction displayed by heart rate is eliminated when the data are converted to heart period. It appears that a considerable portion of the resultant interaction between sympathetic and parasympathetic innervations of the heart may arise simply from use of a nonlinear metric to index chronotropic control.

**Empirical evidence of sympathetic/parasympathetic interactions.** Several studies of the resultant sympathetic-parasympathetic interaction on chronotropic control provide
data for a comparison between heart period and heart rate. These data are summarized in Table 1.
Figure 6. Interactions illustrated by the equation of Levy and Zieske (1969). Large panels indicate change scores from basal heart rate or heart period. Insets show absolute heart rate or period data in bpm or msec. **Left panel:** This panel depicts heart rate data across the range of vagal stimulation used by Levy and Zieske (1969) for 5 levels of sympathetic activation. The data were modeled using the regression equation of Levy and Zieske (1969) that includes a polynomial interaction term. **Right panel:** This panel illustrates the conversion of the heart rate data of the left panel to heart period. The data in the right panel indicate that no interaction remains when heart rate data are converted to heart period.
Figure 6.
Table 1. Effects of single and combined stimulations of sympathetic and vagal cardiac efferents on heart rate and heart period.

### Heart Rate

<table>
<thead>
<tr>
<th>Study</th>
<th>Basl</th>
<th>Vagal stim</th>
<th>Symp stim</th>
<th>Combined Predicted</th>
<th>Combined Observed</th>
<th>Error&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stramba-Badiale et al., 1991</td>
<td>120</td>
<td>70 bpm/-50</td>
<td>201 bpm/+81</td>
<td>151 bpm/+31</td>
<td>95 bpm/-25</td>
<td>2.24</td>
</tr>
<tr>
<td>Levy &amp; Zieske, 1969&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181</td>
<td>100 bpm/-70</td>
<td>260 bpm/+78</td>
<td>189 bpm/+8</td>
<td>120 bpm/-70</td>
<td>1.11</td>
</tr>
<tr>
<td>Figure 2</td>
<td>180</td>
<td>105 bpm/-75</td>
<td>262 bpm/+82</td>
<td>187 bpm/+7</td>
<td>106 bpm/-74</td>
<td>1.09</td>
</tr>
<tr>
<td>Urthaler et al., 1986</td>
<td>147</td>
<td>116 bpm/-31</td>
<td>203 bpm/+56</td>
<td>172 bpm/+25</td>
<td>137 bpm/-10</td>
<td>3.50</td>
</tr>
<tr>
<td>Furukawa &amp; Levy, 1984</td>
<td>111</td>
<td>38 bpm/-70</td>
<td>149 bpm/+35</td>
<td>146 bpm/+35</td>
<td>40 bpm/-71</td>
<td>1.49</td>
</tr>
<tr>
<td>Furukawa &amp; Levy, 1983&lt;sup&gt;a&lt;/sup&gt;</td>
<td>130</td>
<td>53 bpm/-77</td>
<td>179 bpm/+49</td>
<td>102 bpm/-28</td>
<td>64 bpm/-66</td>
<td>1.42</td>
</tr>
<tr>
<td>Young dogs</td>
<td>139</td>
<td>90 bpm/-49</td>
<td>174 bpm/+35</td>
<td>125 bpm/-14</td>
<td>97 bpm/-42</td>
<td>1.33</td>
</tr>
</tbody>
</table>

### Heart Period

<table>
<thead>
<tr>
<th>Study</th>
<th>Basl</th>
<th>Vagal stim</th>
<th>Symp stim</th>
<th>Combined Predicted</th>
<th>Combined Observed</th>
<th>Error&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stramba-Badiale et al., 1991</td>
<td>520</td>
<td>960 ms/+440</td>
<td>301 ms/-219</td>
<td>741 ms/+221</td>
<td>681 ms/+161</td>
<td>-0.37</td>
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<tr>
<td>Levy &amp; Zieske, 1969&lt;sup&gt;a&lt;/sup&gt;</td>
<td>331</td>
<td>600 ms/+247</td>
<td>231 ms/-99</td>
<td>479 ms/+148</td>
<td>500 ms/+184</td>
<td>0.20</td>
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<tr>
<td>Figure 2</td>
<td>333</td>
<td>571 ms/+238</td>
<td>229 ms/-104</td>
<td>467 ms/+134</td>
<td>566 ms/+233</td>
<td>0.62</td>
</tr>
<tr>
<td>Urthaler et al., 1986</td>
<td>408</td>
<td>519 ms/+111</td>
<td>321 ms/-87</td>
<td>432 ms/+24</td>
<td>476 ms/+68</td>
<td>0.65</td>
</tr>
<tr>
<td>Furukawa &amp; Levy, 1984</td>
<td>541</td>
<td>1587 ms/+1031</td>
<td>402 ms/-124</td>
<td>1448 ms/+907</td>
<td>1525 ms/+984</td>
<td>0.08</td>
</tr>
<tr>
<td>Adult dogs</td>
<td>462</td>
<td>1132 ms/+670</td>
<td>334 ms/-128</td>
<td>1004 ms/+542</td>
<td>938 ms/+476</td>
<td>-0.14</td>
</tr>
<tr>
<td>Young dogs</td>
<td>432</td>
<td>667 ms/+235</td>
<td>345 ms/-87</td>
<td>580 ms/+148</td>
<td>618 ms/+186</td>
<td>0.20</td>
</tr>
</tbody>
</table>

<sup>a</sup> The baseline score reported here is the mean basal heart rate or period. Change scores, however, are computed from actual pre-stimulation basal heart rate or heart period values given in the text or in figures. Thus, change scores do not always correspond to the mean baseline values above.

<sup>b</sup> Right vagal and right stellate stimulations.

<sup>c</sup> Error scores were computed from the following equation: Combined Observed change score - Combined Predicted change score / Combined Observed change score
The data of Table 1 illustrate heart rate data from studies of cardiac nerve and ganglion stimulation in dogs. A sizeable deviation of the observed heart rate was apparent at the highest level of combined vagal and sympathetic stimulation, relative to that predicted by a simple addition of the single vagal and sympathetic stimulation. These discrepancies reflect the interaction effect. The extent of this deviation was summarized by an error index (Combined Observed change score - Combined Predicted change score / Combined Observed change score) that illustrates the extent and nature of the interaction. For heart rate, error index values approaching zero indicate no interaction, positive numbers indicate a vagally dominant interaction, and negative numbers a sympathetically dominant interaction. The error values for heart rate (mean ± SEM = 1.74 ± .33) uniformly indicate a significant, vagally dominant interaction effect (t = 5.27, df = 6, p < .01). The lower panel of Table 1 illustrates these same data converted to heart period. Error index values approaching zero again indicate no interaction, positive numbers indicate a sympathetically dominant interaction, and negative numbers indicate a vagally dominant interaction. In contrast to heart rate error indices, the heart period error values are not significantly different from zero, and are directionally inconsistent (mean ± SEM: 0.15 ± 0.13; t = 1.15, df = 6, p > .20) indicating a minimal overall interaction of sympathetic
and parasympathetic innervations on cardiac chronotropism.

**Interactions and the autonomic space equation.** The data outlined above suggest that although there may be complex bidirectional interactions between the sympathetic and parasympathetic innervations of the heart, the overall interaction effect on cardiac chronotropism may be minimal. Rather, much of the apparent interaction with heart rate may derive from the level dependency inherent in rate measures.

For purposes of the autonomic space model for the rat, it may suffice to set the interaction term to null until more is known about the extent of the net interaction of autonomic innervations on cardiac chronotropic control. Indeed, for three corners of autonomic space, where either or both sympathetic or parasympathetic activation is zero, no interaction would be possible. Consequently, interactions may not seriously distort the autonomic space model of cardiac chronotropism.

In order to map basal loci or phasic movements in autonomic space, it is necessary to quantify the separate contributions of the autonomic branches to basal state and phasic heart period response. The use of pharmacologic autonomic blockades provides a useful means of deriving the necessary parameters for creating and utilizing autonomic space depictions. In the next chapter, I will demonstrate
quantitative procedures by which sympathetic and parasympathetic estimates can be obtained from autonomic blockades, and by which validity metrics for these estimates can be derived.
Quantitative autonomic space analyses require specification of the sympathetic and parasympathetic contributions to basal state and reactive response (i.e. empirical determinations of $S_i$ and $P_j$). Among the most useful means of deriving sympathetic and parasympathetic estimates for the conscious, freely moving subject is by pharmacologic autonomic blockade. Other potential sources of these estimates might derive from non-invasive indices, or selective target organ denervations. Pharmacologic blockade is not free of potential biases, however, and the need arises for quantitatively estimating sympathetic and parasympathetic contributions, as well as the extent of bias in autonomic blockade data.
Quantification of the extent of bias in autonomic estimates

Estimates of autonomic control derived from autonomic blockades can be systematically biased by several sources, including interactions between the branches at the level of the target organ, indirect or reflexive alterations in the unblocked branch, nonselective actions of the blocker agents, and incomplete blockades. Some of these biases can be minimized methodologically by careful selection of antagonists and doses to maximize cardioselectivity, and to ensure relatively complete blockades. Biases such as potential reflex alterations in the unblocked branch, however, may not be readily controllable. Moreover, blockade with competitive antagonists is always relative, and there is generally a dose tradeoff between completeness and selectivity. These residual biases can distort the results, and must be quantified to ensure the validity of autonomic estimates derived from blockade studies.

Sympathetic and parasympathetic estimates derived from blockade studies. Autonomic blockade studies utilize sympathetic and parasympathetic blockades to extract estimates of sympathetic and parasympathetic contributions to an observed, unblocked response or tonic level. A given blockade, in fact, provides an estimate of both autonomic branches (Berntson, Cacioppo & Quigley, submitted).
best illustrated by considering the phasic response, as depicted in Figure 7B. The change in response after a single blockade reflects the subtractive loss of that branch. A subtractive estimate ($s''$ or $p''$) of the normal contributions of the blocked branch can be derived from the difference between the observed response (relative to pre-stimulus baseline) in the unblocked and blocked conditions. Here, a given autonomic antagonist yields an estimate of the normal contribution of the blocked branch by the subtractive loss of the response after blockade. In contrast, the remaining autonomic control after blockade of one autonomic branch provides a residual estimate ($s'$ or $p'$) of the contribution of the unblocked branch. Similar estimates can be derived for absolute chronotropic level (Figure 7A), although in this case, the reference point is the intrinsic heart period ($\beta$) instead of the pre-stimulus baseline (Berntson, Cacioppo & Quigley, submitted). The subtractive estimates, therefore, are derived from blockade of the target autonomic branch to be estimated, with reference to the unblocked tonic or phasic level, whereas the residual estimates are derived from blockade of the alternate branch and are referenced to the intrinsic heart period or the pre-stimulus baseline (Berntson, Cacioppo & Quigley, submitted). Selective blockades of both autonomic divisions thus provide two separate estimates, subtractive and residual, of the contributions of each autonomic branch.
Figure 7. Illustrative heart period levels and responses obtained under unblocked conditions, and after selective sympathetic and parasympathetic blockades, or dual blockade.

A. Tonic heart period levels. Blockade conditions are listed to the right of the illustrated heart period levels. Solid arrows represent residual-model estimates of autonomic contributions (s' and p'), referenced to the zero point of autonomic control (intrinsic period or \( \beta \)). Dashed arrows depict subtractive-model estimates (s'' and p''), expressed as a change from the unblocked heart period. Heart period levels under sitting and standing approximate values obtained empirically (see Berntson et al., in press).

B. Phasic heart period responses. Responses are depicted as the change from pre-stimulus heart period level associated with the respective blockade condition. Solid arrows again represent residual-model estimates of autonomic contributions to phasic response (s' and p'), referenced to the pre-stimulus heart period. Dashed arrows illustrate subtractive-model estimates of autonomic contributions to phasic response (s'' and p''), expressed as a difference from the observed, unblocked heart period response.
A. Tonic ANS Levels

<table>
<thead>
<tr>
<th></th>
<th>Sitting</th>
<th>Standing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s' = s&quot; = -100 ms</td>
<td>s' = s&quot; = -160 ms</td>
</tr>
<tr>
<td></td>
<td>p' = p&quot; = 400 ms</td>
<td>p' = p&quot; = 300 ms</td>
</tr>
</tbody>
</table>

B. Phasic ANS Response

<table>
<thead>
<tr>
<th></th>
<th>Prestimulus Basl</th>
<th>Poststimulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Obs. = 75 ms</td>
<td>Prestimulus Basl</td>
</tr>
<tr>
<td></td>
<td>s' = s&quot; = 50 ms</td>
<td>Pp' = p&quot; = 25 ms</td>
</tr>
<tr>
<td></td>
<td>Observed</td>
<td></td>
</tr>
</tbody>
</table>

Figure 7.
Because the subtractive and residual estimates of a given branch are derived from distinct blockade conditions, they are differentially sensitive to potential systematic biases that can arise from pharmacological blockades. In the absence of systematic error, there would be no difference between the prime and double prime estimates of sympathetic and parasympathetic contributions to a heart period level or response (Berntson, Cacioppo & Quigley, submitted). However, as is apparent in Figure 7B, any bias in the residual estimate derived from a given blockade condition (e.g. s') would necessarily yield an inverse bias in the subtractive estimate (p") derived from the same blockade condition (and vice versa). Thus, systematic biases in blockade studies manifest in discrepancies between the two (residual and subtractive) estimates of a given autonomic branch, as derived from alternate blockade conditions. Moreover, because the prime and double prime estimates for each branch are derived from different blockade conditions, these two estimates are necessarily inversely biased. The discrepancies between these alternate derivations, therefore, permit a quantitative specification of the magnitude of biases in blockade data (Berntson, Cacioppo & Quigley, submitted).

In the absence of additional information, the best estimate of the separate contributions of the autonomic branches is the mean of the prime and double prime
estimates, i.e. \( s_{\text{mean}} = (s' + s'')/2 \) and \( p_{\text{mean}} = (p' + p'')/2 \). Moreover, because of the inverse symmetry between the prime and double prime autonomic estimates, the error bias in these estimates can be shown to be: \( E_{\text{blk}} = (s' - s'')/2 = (p' - p'')/2 \) (Berntson, Cacioppo & Quigley, submitted). The magnitude of bias thus provides a validity metric which indexes the systematic discrepancies between the prime and double prime estimates for each branch (see Berntson, Cacioppo & Quigley, submitted). When the magnitude of this error is small with respect to the size of the phasic response then valid interpretations can be made from autonomic blockade data.

A similar approach yields estimates of absolute sympathetic and parasympathetic levels, and permits a parallel validity index for these estimates (Berntson, Cacioppo & Quigley, submitted). In contrast to the phasic response, however, analysis of tonic levels requires an estimate of \( B \) for deriving residual estimates. Because \( B \) estimates are biased by both blockade conditions, absolute estimates have a larger error term than sympathetic and parasympathetic estimates derived from single blockades.
Example of the use of residual and subtractive estimates: Tonic movements on the autonomic surface

A significant shift in tonic, autonomic outflows to the heart occurs upon assumption of an upright posture from supine (Spyer, 1990; Weise, Heydenreich, & Runge, 1987). Upon standing, the arterial baroreceptors are unloaded, resulting in a decrease in tonic activation of afferents to the nucleus tractus solitarius. This results in a baroreflexive reduction in vagal, and increase in sympathetic traffic to the heart and vasculature. The reciprocal mode of autonomic control invoked by orthostatic manipulations results in a progressive shortening of basal heart period from supine to sitting to standing (Nyberg, 1981; Robinson, et al., 1966; Spyer, 1990; Weise et al., 1987).

To demonstrate the utility of autonomic and error bias estimates, and autonomic space depictions, the studies of Nyberg (1981) and Robinson et al. (1966) were used to illustrate tonic movements along the autonomic surface with changes in posture. These studies are useful because they employed near complete sympathetic, vagal, and combined autonomic blockade, and used three orthostatic manipulations ranging from supine to standing posture (or roughly equivalent tilt angles). The values used to populate the autonomic surface are summarized in Table 2. Figure 8 depicts the autonomic effects of postural alterations within
autonomic space as derived by the methods of Berntson, Cacioppo and Quigley (submitted). The autonomic space illustrates the essentially reciprocal autonomic control of heart period with progressive loading or unloading of the baroreceptors. These studies provide relatively unbiased estimates of sympathetic and parasympathetic contributions to these postural states as noted by the small errors with respect to mean sympathetic and parasympathetic estimates. Moreover, these studies demonstrate the use of autonomic space analyses in illuminating tonic autonomic state within the full range of autonomic control.
Table 2. Mean sympathetic and parasympathetic contributions to tonic heart period with postural alterations. The postural data of Nyberg (1981) and Robinson et al. (1966) are summarized, along with the subtractive (double prime) and residual (prime) sympathetic and parasympathetic estimates, and error estimates. Values are expressed in msec of heart period.

<table>
<thead>
<tr>
<th>Source</th>
<th>Posture</th>
<th>$s'/s''$</th>
<th>$s$</th>
<th>$p'/p''$</th>
<th>$E$</th>
<th>$E_{bl}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robinson et al. (1966)</td>
<td>80° tilt</td>
<td>-191/-181</td>
<td>-171</td>
<td>314/304</td>
<td>309</td>
<td>± 10</td>
</tr>
<tr>
<td>Robinson et al. (1966)</td>
<td>45° tilt</td>
<td>-157/-136</td>
<td>-147</td>
<td>400/421</td>
<td>411</td>
<td>± 21</td>
</tr>
<tr>
<td>Nyberg (1981)</td>
<td>Supine</td>
<td>-103/-54</td>
<td>-79</td>
<td>446/495</td>
<td>471</td>
<td>± 49</td>
</tr>
<tr>
<td>Robinson et al. (1966)</td>
<td>0° tilt</td>
<td>-58/-118</td>
<td>-88</td>
<td>598/538</td>
<td>568</td>
<td>± 60</td>
</tr>
</tbody>
</table>
Figure 8. Tonic movements across the autonomic surface. Postural manipulations in human subjects from supine (▼) to sitting (♦) to standing (▲) for the studies of Nyberg (1981, closed symbols) and Robinson et al. (1966, open symbols). Due to considerable individual differences in intrinsic heart period in humans, heart period values on the z-axis are illustrated as a msec change from the intrinsic period (HPa). (Note: Validity error bars (Ebik) are sufficiently small to be hidden by the symbols.) Vertical lines are projected onto the autonomic plane from loci on the autonomic surface to illustrate the reciprocal mode of control as movements along, or parallel to the reciprocity diagonal on the autonomic plane.
Discussion

The model and quantitative analyses derived above will guide analyses of phasic cardiac chronotropic control following pharmacological and behavioral manipulations in subsequent chapters. Following the general methods in Chapter 4, the effects of the benzodiazepine receptor partial inverse agonist, FG 7142 on the cardiovascular response to a nonsignal auditory stimulus will be presented in Chapter 5. Chapter 6 will detail the effects of classical conditioning on autonomic and cardiovascular responses to nonsignal and signal stimuli. Finally, in Chapter 7, I will discuss the pragmatic benefits and conceptual insights provided by the autonomic space model.
CHAPTER IV
GENERAL METHODS

This chapter details the subjects, instrumentation, pharmacologic agents and general experimental context for the studies reported in Chapters 5 and 6.

Subjects

Subjects were male Sprague-Dawley rats (Zivic Miller, Zelienople, PA) maintained on a 12 hr light/dark cycle with testing during the first half of the light cycle. Subjects had ad libitum access to food and water. For 2 to 6 weeks prior to experimental procedures, subjects were handled for 5 min/day, 2-3 days/week. Handling included being picked up by the shoulders, stroking the head and flank, and light pinches of the abdomen to simulate i.p. injections.

Surgical procedure

Subjects were instrumented under anesthesia with a chronic catheter implanted into the right common carotid artery for measurement of heart period and blood pressure. The silastic catheter was attached to a septum-covered reservoir with an affixed collar that was exteriorized at
the neck (Vascular Access Port, Access Technologies, Skokie, IL). Following anesthesia with ketamine and xylazine (90 mg/kg and 6 mg/kg, i.p., respectively), the animal was shaved and incisions made in the ventral and dorsal cervical areas. The vagus and other nerve fibers were carefully separated from the carotid artery and the artery was ligated rostrally. An incision was made in the arterial wall and the tip of the catheter (0.51 mm i.d. X 0.84 mm o.d.) was advanced to the level of the aortic arch and secured. The vascular access port was exteriorized at the dorsum. Heparinized saline (10 USP units/cc) was administered via the catheter during surgery and 2-4 times per day thereafter (0.1-0.3 ml bolus). Subjects were administered dextrose or saline, and penicillin (15 mg in 0.3 cc, i.p., and 60,000 units in 0.2 cc, i.m., respectively) immediately following surgery. Animals were allowed 24 hours recovery before testing.

Autonomic blockade

Sympathetic autonomic blockade was effected by the $\beta_1$-adrenergic antagonist, atenolol, a relatively "cardio-selective" agent without intrinsic sympathomimetic or membrane-stabilizing properties, and having low lipophilicity (Cruickshank, 1980; Frishman, 1981; Minneman, Hegstrand & Molinoff, 1979). Parasympathetic blockade was accomplished using the quaternary, muscarinic cholinergic
antagonist, scopolamine methylnitrate to minimize diffusion into the brain (Gilman, Goodman, Rall & Murad, 1985; but see Moore, Dudchenko, Comer, Bruno & Sarter, 1992). Dosages are reported in the methods sections for each study.

**Experimental context and recording apparatus**

Subjects in the study reported in Chapter 5 were tested in a shaving-lined chamber (51L X 25W X 30H cm) within a sound-attenuated, electrically shielded recording chamber (Industrial Acoustics, Inc., Bronx, NY). Subjects in conditioning experiments (Chapter 6) were tested in a Plexiglas conditioning chamber (28L X 21.5W X 51H cm) within the shielded recording chamber. The conditioning chamber had a drop-front door and a floor of stainless steel bars placed 1.5 cm apart (center to center). A tray beneath the shock grid was emptied and cleaned between subjects. A background white noise stimulus (50 db SPL) was presented continuously for all subjects. The background and other auditory stimuli were presented by a free-field speaker located above the subject.

Blood pressure was recorded via a pressure transducer (Model TNF-R, Columbus Instruments, Columbus, OH) which was coupled to a polygraph (Model 7, Grass Instruments, Quincy, MA) for signal amplification and filtration (DC to 15 Hz). The analog signal was then passed to a computer for A/D
conversion (500 Hz, 12 bit) and storage of the digitized data for offline processing. A calibration pressure signal was digitized and stored with all data. Heart period was derived by a computerized peak-finding algorithm from the pulse wave of the pressure signal. Unless otherwise noted, mean heart period and mean arterial pressure were derived over 30 second periods for tonic measures, and on a second by second basis for phasic measures.

**Data reduction and statistical analysis**

Data were examined both visually and with a computerized algorithm for identification and correction of movement or recording artifacts (Berntson, Quigley, Jang, & Boysen, 1990). Trials were removed from the analysis if the prestimulus baseline was unstable, or if excess movement artifacts were apparent. The percentage of data removed is specified for individual studies and never exceeded 8%.

Analysis of variance was used to examine the effects of pharmacological and psychological manipulations on basal and reactive heart period and blood pressure. Phasic cardiovascular responses were quantified by deriving the integral area under the heart period or blood pressure response relative to the pre-stimulus baseline. Post hoc comparisons were made using Fisher's least significant difference method.
CHAPTER V
EFFECTS OF THE BENZODIAZEPINE RECEPTOR INVERSE AGONIST, FG 7142 ON TONIC AND PHASIC CARDIOVASCULAR FUNCTION

FG 7142 (N'-methyl-β-carboline-3-carboxamide) has been classified as a benzodiazepine receptor (BZR) partial inverse agonist on the basis of its ability to inhibit GABAergic transmission (Braestrup, Schmiechen, Neef, Nielsen & Peterson, 1982), to exert proconvulsant effects at relatively high doses (Jensen, Peterson & Braestrup, 1983), and to exert proconflict effects (Petersen, Paschelke, Kehr, Nielsen & Braestrup, 1982; Petersen and Jensen, 1984). However, in contrast to BZR full inverse agonists, FG 7142 does not produce seizures when given acutely.

The proconflict effects of FG 7142 shown in earlier animal experiments have been assumed to reflect drug-induced stress or anxiety. This hypothesis has become a subject of intense debate since the report on the effects of this drug in human volunteers (Dorow, Horowski, Paschelke, Amin & Braestrup, 1983). Although the findings from this open, uncontrolled trial may not represent a valid basis for
conclusions about the anxiogenic properties of FG 7142 (see Thiebot, Soubrie & Sanger, 1988), subsequent animal studies have supported the hypothesis that this drug produces behavioral effects which mimic anxiety. This provides some face validity for the view that FG 7142 may be anxiogenic (e.g., Beck and Cooper, 1986a, b; Thiebot, Dangoumau, Richard & Puech, 1991; Piret, Depaulis & Vergnes, 1991).

Several studies have suggested the possibility that the anxiogenic or stressor-like effects of FG 7142 may be mediated via a selective increase in metabolic activation of the dopaminergic prefrontal cortical input, a result consistent with the effects of environmental stressors (Moghaddam, Roth & Bunney, 1990; Knorr, Deutch & Roth, 1989). In addition, GABAergic systems are modulated directly by BZR ligands, and in turn, cholinergic systems are modulated via GABAergic inputs. Both neurotransmitter systems have been implicated in the response to stress (Coco, Kuhn, Ely & Kilts, 1992; Dilsaver, 1988; Gilad, 1987; Ray, Henke & Sullivan, 1990; Weiss, Goodman, Losito, Corrigan, Charry & Bailey, 1981).

Although BZR partial inverse agonists appear to exert anxiogenic behavioral effects, their potential cardiovascular and autonomic actions have not been fully characterized. Previous research suggests that increased cardiovascular and autonomic reactivity may correlate with the response to anxiety- or fear-producing stimuli (Iwata &
LeDoux, 1988; Quigley & Berntson, 1990; Smith, Astley, DeVito, Stein & Walsh, 1980). Moreover, significant neuroanatomical and biochemical links exist between systems implicated in cardiovascular regulation and emotional response (LeDoux, 1987). The BZR inverse agonist, B-CCE (ethyl-β-carboline-3-carboxylate), for example, has been reported to elevate heart rate and blood pressure in animals, as well as increase plasma cortisol and catecholamines, consistent with the putative anxiogenic actions of this compound (Crawley et al., 1985; Ninan, Insel, Cohen, Cook, Skolnick & Paul, 1982; Skolnick, Ninan, Insel, Crawley & Paul, 1984). Little data exist, however, on the cardiovascular effects of FG 7142. An ex vivo study of the isolated rat heart revealed that FG 7142 has no direct effects on either the inotropic or chronotropic state of the heart, nor does it alter the cardiac response to norepinephrine (Stanford, Gettins & Little, 1990). Dorow et al. (1983) reported that, in one human subject, administration of 200 mg FG 7142 resulted in an increase in blood pressure (from 105/50 to 160/100 mmHg) and pulse rate (from 80 to 110 bpm). These effects were accompanied by reports of severe anxiety and agitation. Although these initial reports are intriguing, the cardiovascular actions of FG 7142 have yet to be fully explored.

A comprehensive characterization of the potential cardiovascular effects of this agent requires more than a
simple cataloging of effects on cardiovascular target organ responses. As is apparent from the discussion of autonomic analyses, the functional output of a dually-innervated target organ such as heart is equivocal with respect to its underlying autonomic origins.

The present experiment investigated the effects of the BZR partial inverse agonist FG 7142 on basal cardiovascular state and on reactive response to a nonsignal auditory stimulus. In addition, the autonomic origins of the cardiovascular outcomes were assessed by selective pharmacological blockade of the independent sympathetic and parasympathetic contributions.

Methods

Subjects, physiological recordings, and stimuli

Subjects were 12 male Sprague-Dawley rats weighing 475 ± 71 g (mean ± SD). Subjects were maintained and instrumented for cardiovascular recordings as noted in the General Methods. Heart period and blood pressure were recorded in 30 sec trials as previously noted. Auditory stimuli were square-wave pulses (1000 Hz, 20 sec duration) presented at 60 db (SPL).
Pharmacological agents and experimental regimen

The dose of the benzodiazepine receptor inverse agonist FG 7142 (8 mg/kg, i.p., suspended in a volume of 10% cremofoer EL (BASF, Ludwigshafen, Germany) equivalent to 0.1% body mass) was chosen on the basis of a pilot dose response study and from data in the literature, to yield measurable cardiovascular actions, while minimizing the likelihood of proconvulsant activity. Although previous studies demonstrated that electrocortical records evidenced epileptogenic activity at 30 mg/kg, electrocortical patterns were unaffected by more moderate doses of FG 7142 (3 or 10 mg/kg; Stutzmann, Bohme, Cochon, Roux & Blanchard, 1987). The dosage employed is within the reported range for the induction of putative anxiogenesis (Petersen and Jensen, 1984; Petersen et al., 1982). Finally, pilot doses of FG 7142 did not yield noticeable effects on the general behavior of the subjects. Autonomic blockade was effected by atenolol (1 mg/kg, s.c.) and scopolamine methylnitrate (0.1 mg/kg, s.c.) in a concentration of 0.1 mg/ml.

Each subject received FG 7142 (or its vehicle) followed by an autonomic antagonist (or saline) in a 2 X 2 design. Subjects were divided into two groups of six, matched for reactivity to the auditory stimulus under saline conditions, with each group receiving one of the two autonomic blockers. Each subject received the following pre-treatment/treatment
regimen in block-randomized order over 4 days: (1) vehicle, saline; (2) vehicle, autonomic blockade; (3) FG 7142, saline; (4) FG 7142, autonomic blockade. A 10 min adaptation period began each session immediately followed by three 30 sec baseline trials. FG 7142 or its vehicle was administered immediately following the third baseline trial, and 20 min later, the autonomic antagonist or saline was administered. Ten min after this second injection, 3 drug baseline trials were recorded, followed by 6 presentations of the auditory stimulus at a variable 2 min ITI.

Data reduction and analysis

Data reduction procedures as outlined in the General Methods resulted in removal of less than 8% of the data. Cardiovascular responses to the auditory stimulus were quantified by deriving the integral area under the pre- and post-stimulus heart period and blood pressure response curves over three, 9 sec time blocks (1 pre- and 2 post-stimulus blocks). These data were then submitted to repeated measures ANOVAs to examine the effects of FG 7142 on reactive heart period and blood pressure. Because separate analyses were conducted for pressor and depressor components of the blood pressure response, the p value for these analyses was adjusted to 0.025.
Results

Effects of FG 7142 on basal heart period and blood pressure

FG 7142 resulted in a moderate increase in basal heart period, but did not alter basal blood pressure as evidenced by the results of Time (Pre/Post) X Drug (Vehicle/FG 7142) ANOVAs on mean heart period and blood pressure. The FG 7142-induced increase in heart period was indicated by a significant Time X Drug interaction. Post hoc tests revealed that heart period was significantly increased following FG 7142 (mean post-vehicle = 175.6 ± 4.3; mean post-drug = 192.8 ± 6.0; Interaction: F (1, 11) = 6.29, p < .05; Post-vehicle vs. post-drug comparison: p < .05). Conversely, FG 7142 did not alter basal blood pressure as evidenced by a lack of main effects or interactions (mean post-vehicle = 111.1 ± 2.8; mean post-drug = 108.0 ± 2.3; Drug main effect and interaction: Fs < 1.0, ps > .3).

Effects of FG 7142 on phasic heart period and blood pressure responses

Reactive heart period and blood pressure responses to the auditory stimulus were submitted to separate Time (Pre/Post1/Post2) X Drug (Vehicle/FG) repeated measures ANOVAs. Because virtually all cardiac responses were monophasic and acceleratory, analyses were based on integral areas under the cardioacceleratory heart period response
function\(^1\). A significant acceleratory response to the stimulus was revealed by a significant main effect of Time \((F (2, 22) = 162.07, p < .001)\). Post hoc analyses revealed that reactive heart period responses following both vehicle and FG 7142 pre-treatment were significantly different from baseline (all Pre vs. Post1 or Post2 comparisons significant; \(p < .01\)). Furthermore, the response to the auditory stimulus after both FG 7142 and vehicle pre-treatment was relatively stable over the two post-stimulus time blocks (Post1 vs. Post2: \(p > .05\)). Reactive heart periods following FG 7142 were enhanced relative to vehicle controls as revealed by a significant main effect of Drug \((F (1, 11) = 16.13, p = .002; \text{Figure 9})\). These results demonstrate that the tachycardiac response to the auditory stimulus is significantly enhanced by FG 7142.

\(^{1}\) Because the assumption of homogeneity of variance was violated by the reactive heart period integral areas, analyses were performed on log-transformed integrals. Ancillary analyses on untransformed heart period integrals revealed results comparable to those obtained with log-transformed heart periods for the effects of both FG 7142 and autonomic blockade.
Figure 9. Heart period and blood pressure responses to nonsignal stimuli in vehicle or FG 7142 pre-treated rats. Mean data (n = 12) are presented as changes from pre-stimulus baseline values. The solid vertical line indicates stimulus onset. Data illustrate that the FG 7142 pre-treatment heart period and depressor responses were enhanced relative to vehicle controls.
Figure 9.

Blood Pressure Response (mmHg)

Heart Period Response (msec)
Subjects exhibited significant pressor and depressor responses following the auditory stimulus (both Time main effect ps < .001). Although pre-treatment with FG 7142 did not alter the pressor response to the auditory stimulus, the stimulus-evoked depressor response was significantly larger than that exhibited after vehicle (Time X FG 7142 interaction: F (2, 22) = 5.24, p < .013; Vehicle post stimulus block 1 vs. FG 7142 post stimulus block 1: p < .01).

Effects of autonomic blockade on basal heart period and blood pressure

Under quiescent conditions, basal heart period in the adult rat is under considerable vagal control (Quigley and Berntson, 1990; Yongue, McCabe, Porges, Rivera, Kelley & Ackles, 1982). Consistent with this, vagal blockade by scopolamine resulted in a substantive decrease in basal heart period (mean baseline heart period = 170.0 ± 7.5; mean scopolamine heart period = 131.7 ± 2.1). This was revealed by a significant Scopolamine main effect (F (1, 5) = 36.61, p = .002), and a significant Time X Scopolamine interaction (F (1, 5) = 105.79, p < .001) reflecting the difference between pre- and post-scopolamine heart period. Conversely, although sympathetic blockade with atenolol lengthened heart period somewhat, this difference did not achieve
significance (mean baseline heart period = 181.2 ± 9.2; mean atenolol heart period = 192.7 ± 5.2; ps > .3).

The sympathetic system exerts control over vascular smooth muscle via α-adrenergic receptors, and the β₁ antagonist (atenolol) used in the present study would be expected to have minimal effect on the vasculature. In fact, neither scopolamine nor atenolol significantly altered mean blood pressure (all ps > .2).

Effects of autonomic blockade on phasic heart period and blood pressure responses

Because the cardiac response to the auditory stimulus was sustained throughout the post-stimulus interval, analyses of the effects of autonomic blockade were performed using the integral areas under the entire post-stimulus response².

Effects of autonomic blockade on control responses. Blockade of β₁-adrenergic receptors by atenolol resulted in a significant reduction in the reactive heart period response following vehicle pre-treatment (Atenolol main effect: F (1, 5) = 9.36, p < .03; Figures 10 & 11). Thus,

² As with analyses of the effect of FG 7142, heart period integral area measures were log transformed to normalize the data. Ancillary analyses on untransformed integral areas produced results comparable to those of the log transformed integrals.
the cardioacceleratory response observed after vehicle pre-
treatment was at least partially attributable to sympathetic
activation. Similarly, scopolamine methylnitrate also
revealed a significant reduction of the cardioacceleratory
response (Scopolamine main effect: $F(1, 5) = 23.40, p =
.005$; Figure 10 & 11). Taken together, these overall data
would suggest a reciprocal mode of control underlying the
cardiac response following vehicle pre-treatment. However,
when considering only the response (exclusive of the
recovery to baseline) using fine-grained analyses, the data
suggest, instead, a prominent initial coactivation followed
by parasympathetic uncoupled withdrawal (cf. Figures 10 &
12). Such subtle alterations in control are not captured by
a bar graph illustrating gross changes in the heart period
response.
Figure 10. Effects of vehicle or FG 7142 pre-treatment and autonomic blockade on the integral area under the post-stimulus acceleratory heart period response. For each panel \( N = 6 \). The data portray the significant attenuation of the post-stimulus response by both atenolol and scopolamine after either vehicle or FG 7142 pretreatment.
Figure 10.
Figure 11. Auditory stimulus-evoked heart period responses following vehicle pre-treatment and autonomic blockade, and derived sympathetic and parasympathetic response estimates. **Upper panel:** Heart period responses after vehicle pre-treatment and administration of saline, $\beta_1$-adrenergic blockade (atenolol), or muscarinic blockade (scopolamine). **Lower panels:** Estimates of sympathetic and parasympathetic contributions to the heart period response and corresponding validity intervals.
Figure 11.
Figure 12. Autonomic space depiction of the heart period response to the auditory stimulus after vehicle pre-treatment. The expanded insert depicts the time-varying cardiac response as movements along the sympathetic and parasympathetic axes. For illustration, the insert axes units are expressed as msec change in heart period. The beginning point for the response is placed for illustration at 0, 0 and lines extending from this basal starting point depict the temporally unfolding response. Data points illustrate the change in response at 1 second intervals, and for clarity, the final 2 post-stimulus seconds were omitted. A prominent initial coactivation followed by a uncoupled parasympathetic withdrawal appears to underlie the heart period response (excluding the recovery of the response toward baseline).
As outlined above, the results of the separate autonomic blockades provide estimates of the independent contributions of the autonomic branches to the observed heart period response. The mean of the subtractive and residual estimates for each autonomic branch are illustrated in Figure 11, together with the validity ranges as derived by the method of Berntson, Cacioppo and Quigley (submitted). Minimal discrepancies were apparent in the sympathetic and parasympathetic estimates illustrated in Figure 11.

In contrast to heart period, analyses revealed no effect of either scopolamine or atenolol on the pressor or depressor responses to the auditory stimulus in vehicle pre-treated subjects, either for the first post-stimulus block or for the entire post-stimulus period (all ps > .1).

**Effects of autonomic blockade on responses after FG 7142.** Similar to the results after vehicle pre-treatment, atenolol significantly attenuated the response to the auditory stimulus following FG 7142 pre-treatment (Figures 10 & 13), effectively eliminating the FG 7142-potentiated response. This is consistent with an FG 7142-induced increase in sympathetic reactivity. However, muscarinic blockade also nearly abolished the stimulus-evoked cardiac response (Figures 10 & 13). Sympathetic and parasympathetic estimates, and validity intervals were derived, by the methods of Berntson, Cacioppo and Quigley (submitted). As
is apparent in Figure 13, the sympathetic and parasympathetic estimates deviate considerably from the observed autonomic responses. These results indicate a confound in the effects of autonomic blockade under FG 7142 pre-treatment which is not apparent after the vehicle. I will return to the issue of the possible sources of this confound below.

Neither depressor nor pressor responses to the auditory stimulus were altered following autonomic blockade and FG 7142 pre-treatment (all ps > .1).
Figure 13. Auditory stimulus-evoked heart period responses following FG 7142 pre-treatment and autonomic blockade, and derived sympathetic and parasympathetic response estimates. Upper panel: Heart period responses after FG 7142 pre-treatment and saline, β1-adrenergic blockade (atenolol), or muscarinic blockade (scopolamine). Lower panels: Estimates of sympathetic and parasympathetic contributions to the heart period response and corresponding validity intervals.
Figure 13.
Discussion

In freely moving rats the benzodiazepine receptor partial inverse agonist, FG 7142 modestly increased basal heart period, but did not alter mean blood pressure. More notably, FG 7142 dramatically increased both the reactive cardioacceleratory, and depressor responses to a moderate intensity auditory stimulus.

The observed increase in basal heart period following FG 7142 contrasts with findings of decreased heart period after inverse agonist (β-CCE or FG 7142) treatment in primates (Crawley et al., 1985; Ninan et al., 1982; Skolnick et al., 1984). It is not clear if these deviations represent species differences, or procedural variations. The lack of effects of atenolol on the basal heart period change reported here suggests that the modest increase in baseline heart period after FG 7142 pre-treatment likely arose from minimal increases in tonic parasympathetic outflow to the heart. Such a result is consistent with prior evidence of a converse, vagolytic effect of BZR agonists which modulate chloride conductance at the GABA receptor in a direction opposite that of the inverse agonists (Adinoff, Mefford, Waxman & Linnoila, 1992; Conahan & Vogel, 1986; DiMicco, 1987).

In contrast to the modest effects of FG 7142 on basal heart period, the reactive tachycardiac response to an
auditory stimulus was appreciably enhanced after FG 7142 relative to controls. This result is in keeping with an increased stress reactivity following inverse agonist administration, as revealed by increased release of corticosteroids and plasma catecholamines (Crawley, et al., 1985; Ninan, et al., 1982; Pellow & File, 1985; Skolnick et al., 1984). Moreover, the enhanced reactivity after FG 7142 may account for previously reported decreases in basal heart period (e.g. Dorow et al., 1983; Ninan et al., 1982) because these studies were conducted in at least mildly stressful contexts (e.g. drug administration to humans in a laboratory or monkeys in a restraint chair). Thus the basal cardiovascular results of previous studies may partially reflect reactive alterations. These results serve as an important reminder that modulations of GABAergic transmission by the BZR ligands are made against a background of tonic GABAergic activity. Only under minimally evocative experimental conditions will the BZR ligands reveal the effects of modulating true resting GABAergic function.

To further characterize the reactive cardiac response to the auditory stimulus in the present study, sympathetic and parasympathetic cardiac innervations were blocked by atenolol and scopolamine methylnitrate, respectively, and analyzed using the quantitative methods of Berntson, Cacioppo and Quigley (submitted). Based on these analyses,
the cardioacceleratory response to the auditory stimulus in control (vehicle pre-treated) subjects was associated with an initial coactivation followed by a notable parasympathetic withdrawal (considering only the response and not the recovery to baseline; Figure 12). This is consistent with previous results demonstrating a coactivational mode of control in normal rats presented with a comparable auditory stimulus (Quigley & Berntson, 1990).

Sympathetic and parasympathetic blockades were similarly applied to analysis of the autonomic contributions to the cardiac response after FG 7142. In this case, however, considerable discrepancies were apparent in the residual and subtractive estimates, indicating a bias from autonomic blockades. This bias could arise from a number of potential sources, although a distinct possibility is suggested by the literature.

Recent microdialysis evidence suggests that FG 7142 (as well as other BZR inverse agonists) increases release of cortical acetylcholine perhaps due, in part, to the negative modulation by BZR inverse agonists of GABA which normally serves to inhibit ACh release (Moore, Sarter & Bruno, 1992; Moore, Stuckman, Sarter & Bruno, in press). Moreover, central cholinergic systems are known to be heavily involved in behavioral-cardiovascular regulation (Ruggiero, Guiliano, Anwar, Stornetta & Reis, 1990). This raises the possibility that the enhanced cardioacceleratory response to FG 7142 was
related to a central cholinergic potentiation. Moreover, recent evidence suggests that quaternary cholinergic antagonists can penetrate the brain to a functionally significant extent (Moore et al., 1992). Antagonism of an FG 7142-mediated cholinergic potentiation by a central action of scopolamine methylnitrate, for example, could effectively neutralize the cardiovascular actions of FG 7142, and spuriously appear as an exaggerated contribution of the parasympathetic branch to the cardioacceleratory response.

Taken alone, the large response decrement seen after vagal blockade would suggest a major parasympathetic withdrawal underlying the FG 7142-potentiated response. Ancillary analyses, however, indicate that this may represent an overestimate of the parasympathetic contribution. Respiratory sinus arrhythmia (RSA) is a periodic fluctuation in heart period associated with respiration, and is generally regarded as a noninvasive index of vagal control of the heart (Berntson, Cacioppo & Quigley, 1993; Porges, 1986). RSA amplitude measures (natural log of the respiratory band variance) were derived for the post-stimulus cardiac response, after both vehicle and FG 7142 by the method of Porges and Bohrer (1990). As expected, scopolamine largely eliminated RSA, reflecting its parasympathetic origin (Pre-scopolamine baselines: Vehicle = 2.18 ± .18; FG 7142 = 2.13 ± .25; Post-scopolamine
baselines: Vehicle = 0.41 ± .21; FG 7142 = 0.36 ± .39). RSA indices also declined somewhat after stimulus presentations in the unblocked condition, reflecting the parasympathetic withdrawal component of the cardioacceleratory response. The post-stimulus RSA values after vehicle and FG 7142, however, did not appreciably differ (Vehicle = 1.80 ± .11, FG 7142 = 2.04 ± .18), and in fact declined less after FG 7142. These results are not consistent with a substantial parasympathetic withdrawal after FG 7142. Rather, they suggest that scopolamine yielded a biased estimate of autonomic control. This raises the possibility that the mechanism underlying the FG 7142-mediated accentuation of the cardiac response may itself have been blocked by one or both of the autonomic antagonists. Although plausible, the autonomic origins of the FG 7142 cardioacceleratory enhancement remain obscure.

The enhanced cardioacceleratory and depressor responses after FG 7142 may represent components of the psychophysiological response to aversive or anxiety-producing stimuli. This is consistent with our previous results with rats in which the response to a high intensity stimulus consisted of cardioacceleratory and depressor responses that were enhanced relative to those in subjects presented a moderate intensity stimulus (Quigley & Berntson, 1990). Also consistent with previous results was the initial coactivation apparent in vehicle pre-treated
subjects, similar to that of normals in response to a moderate intensity auditory stimulus. As a further test of the generalizability of these results, the next experiment utilized aversive conditioning as a behavioral analog of the putative anxiogenic effects of FG 7142.
CHAPTER VI
EFFECTS OF CLASSICAL CONDITIONING ON SYMPATHETIC
AND PARASYMPATHETIC CONTROL OF HEART PERIOD

To further examine the effects of anxiety on cardiovascular reactivity, I hypothesized that the non-specific aversive responses induced by pseudoconditioning in an aversive conditioning paradigm might provide an anxiogenic behavioral parallel to FG 7142. Classical conditioning represents a simple behavioral paradigm for examining responses to a specific, conditioned aversive stimulus, and to non-specific contextual cues associated with aversive stimulation. Specifically, the hypothesis was that conditioned subjects would display an enhanced cardiovascular and autonomic reactivity to the CS relative to pseudoconditioned subjects, but a cardiovascular response to a nonsignal, probe stimulus similar to nonconditioned subjects. In contrast, pseudoconditioned subjects would exhibit a higher level of general conditioning to contextual, and other stimuli, and display an overall increase in cardiovascular reactivity. Similar to FG 7142, pseudoconditioning would be expected to evince an
enhancement of the cardiac and depressor responses to a moderate intensity auditory stimulus.

To explore these hypotheses, conditioned and pseudoconditioned freely moving rats were presented with CS-US pairs, or random CS-US presentations, respectively, while heart period and blood pressure were recorded. Cardiovascular responses to the CS (extinction trials) and to novel, probe stimuli were recorded to examine the autonomic and cardiovascular effects of discrete, signal and nonsignal stimuli.

**Methods**

**Subjects, physiological recordings, and experimental stimuli**

Subjects were 12 male Sprague-Dawley rats (520 ± 16g, mean ± SEM) maintained and instrumented as noted in the General Methods. Physiological recordings were also made as described above (General Methods), over 30 sec trials. The CS was a 10 sec light (20 W halogen lamp) that illuminated the conditioning chamber through a hole in the side of the chamber. Reflective surfaces (stainless steel/Plexiglas) inside the conditioning chamber permitted the subject to see the light regardless of its position in the chamber. The US was a 2.2 mA footshock presented for the last 0.5 sec of the CS across the grid floor (Model 82400, constant current shock source, Lafayette Instrument Co., Lafayette, IN).
Probe auditory stimuli were 60 db sine-wave pulses similar to those reported in Chapter 5 (1 sec at 1000 Hz with a 50 msec rise/fall). All stimuli and trial onsets were controlled by a microcomputer.

**Pharmacological agents and experimental regimen**

The subjects in the present experiment were pre-treated with the vehicle for FG 7142 (10% cremofor EL) to facilitate future comparisons with FG 7142 and conditioning. Autonomic blockades were effected with scopolamine methylnitrate (0.1 mg/kg, s.c.) and atenolol (10 mg/kg, s.c.).

The day prior to catheter implantation, animals were given a single conditioning session. Subjects were placed in the conditioning chamber, and after a 10 min adaptation period, 10 CS-alone trials were presented at a variable 120 sec ISI (90-150 sec range) to minimize subsequent sensitization to the CS. These habituation trials were followed by 40 presentations of the CS and US. For the conditioning group, the onset of the CS occurred at a variable 120 sec ISI (90-150 sec range), and the US was delivered during the last 0.5 sec of the CS. For the pseudoconditioning group, a similar CS delivery schedule was used, but the US was presented at constrained random intervals.
Twenty four hours after surgery, and for the succeeding 2 days, subjects were tested in the conditioning chamber. Prior to testing, subjects were given a 10 min adaptation period, followed by recording of 3 pre-drug baseline trials for determination of basal heart period and blood pressure. Immediately following baselines, the vehicle pre-treatment was given (0.1% body weight, i.p.) and 20 min later, the autonomic blockade agent or its vehicle (saline) was administered. Ten minutes after administration of the blocking agent, another 3 post-drug baseline trials were recorded. Three paired or random CS-US presentations were given as re-conditioning trials, followed by mixed sequences of 4 extinction trials, 4 auditory probe tones, and 2 final re-conditioning trials. Animals received scopolamine methylnitrate, atenolol and saline counterbalanced across test days.

Data reduction and analysis

Data were reduced and analyzed as noted in the General Methods. Artifacts comprised less than 1% of the data. Cardiovascular responses to either conditioning or probe stimuli were quantified by deriving the integral area under the response curves. Because both heart period and blood pressure responses were occasionally polyphasic, both deceleratory (and depressor) and acceleratory (and pressor) integrals were derived for the entire post-stimulus response
period. These data were then submitted to mixed
between/within subjects ANOVAs to examine the effects of
conditioning and pseudoconditioning on phasic heart period
and blood pressure. Heart period integral measures violated
the assumption of homogeneity of variance, and thus ANOVAs
were conducted on, and reported as log-transformed
integrals.

Results

Effects of conditioning and autonomic blockade on basal
heart period and blood pressure

Basal heart period and blood pressure during extinction
tests did not differentiate training groups (conditioning
(CND) vs. pseudoconditioning (PSD); ps > 0.2; CND mean heart
period = 168.8 ± 7.3; PSD mean heart period = 170.2 ± 5.6;
CND mean blood pressure = 126.4 ± 2.5; PSD mean blood
pressure = 121.0 ± 3.8) in a 2 Training type (CND vs. PSD) X
(3 Drug (Saline, Scopolamine or Atenolol) X 2 Time (Pre- vs.
Post-drug)) mixed between/within ANOVA. Thus, subsequent
baseline analyses were conducted collapsing across training
type. Autonomic blockades altered basal heart period as
revealed by a Drug X Time interaction on basal heart period
(F (2, 22) = 79.32, p < .001) and a main effect of Drug (F
(2, 22) = 48.42, p < .001). Post hoc analyses did not
disclose differences between any pre-drug mean heart periods
(ps > .05), but revealed the expected shortening of basal heart period after vagal blockade with scopolamine (Saline post-drug = 169.5 ± 4.4; Scopolamine post-drug = 130.03 ± 2.3; p < .05). In contrast, atenolol did not significantly alter basal heart period, consistent with the minimal sympathetic tone typically observed under baseline conditions (Atenolol post-drug = 188.2 ± 3.2; p > .05). As expected, mean blood pressure was not affected by autonomic blockades (all Fs < 1.0).

**Effects of conditioning and autonomic blockade on cardiovascular responses**

**Effects of the CS on heart period response during extinction.** The response to the CS was predominantly acceleratory, and ANOVAs on log-transformed acceleratory integrals revealed significant main effects of both Training (CND vs. PSD, F (1, 10) = 11.28, p < .01) and Drug (F (2, 20) = 7.45, p < .005). Under all drug conditions, the acceleratory response to the CS was larger in CND than in PSD subjects (Figure 14; CND mean saline = 2.3 ± 0.2; PSD mean saline = 1.9 ± 0.1). For both CND and PSD subjects, scopolamine reduced the CS-evoked cardioacceleratory response (Figure 15; CND mean scopolamine = 1.7 ± 0.2, PSD mean scopolamine = 0.8 ± 0.2), as did atenolol, a result suggesting that both CND and PSD heart period responses to
the CS arose from an underlying reciprocal mode of control (CND mean atenolol = 1.5 ± 0.4, PSD mean atenolol = 1.1 ± 0.3; Figure 17). Overall autonomic analyses of these responses indicate a low to moderate validity for the derived sympathetic and parasympathetic estimates (Figure 15).
Figure 14. Reactive heart period and blood pressure responses to presentation of the CS (extinction) and to probe auditory stimuli (tone) after conditioning (CND) or pseudoconditioning (PSD). **Left panels:** Heart period (upper panel) and blood pressure (lower panel) responses to extinction trials are illustrated. The vertical line indicates onset of the CS which terminated after 10 sec. **Right panels:** Heart period (upper) and blood pressure (lower) responses to probe tones are depicted. The vertical line indicates tone onset which terminated after 1 sec. Responses in all panels are shown as a change from pre-stimulus baselines.
Figure 14.
Figure 15. Heart period responses to the CS after vehicle pre-treatment and autonomic blockade, and derived sympathetic and parasympathetic response estimates. **Upper panel:** Heart period responses after vehicle pre-treatment and saline, $\beta_1$-adrenergic blockade (atenolol), or muscarinic blockade (scopolamine). **Lower panels:** Estimates of sympathetic and parasympathetic contributions to the heart period response and corresponding validity intervals. Stimulus onset is indicated by the vertical line, and responses are depicted as a change from pre-stimulus baseline.
Figure 15.
Effects of the CS on blood pressure response during extinction. ANOVAs on post-stimulus blood pressure responses revealed significant effects of training on both pressor and depressor responses to the CS (Figure 14; Pressor: $F(1, 10) = 45.22, p < .001$; Depressor: $F(1, 10) = 66.00, p < .001$). Under all drug conditions, depressor responses were larger for PSD subjects than for CND subjects, whereas pressor responses were larger for CND subjects (Figure 14; CND depressor: saline mean = 8.4 ± 3.6; PSD depressor: saline mean = 61.9 ± 11.2; CND pressor: saline mean = 98.6 ± 17.2; PSD pressor: saline mean = 38.9 ± 11.2).

Effects of probe stimuli on heart period response. Responses to probe stimuli were considerably different across subjects, and sometimes polyphasic, therefore, both acceleratory and deceleratory responses were quantified. ANOVAs on acceleratory responses to probe stimuli revealed no significant effects of Training or Drug (Figure 14 & 16; all ps > 0.15). Complementary analyses on deceleratory responses revealed a main effect of Drug that appeared to arise from a decrement in the deceleratory response after scopolamine, and an increment in the deceleratory response after atenolol (CND: saline mean = 1.1 ± 0.4, scopolamine mean = 0.7 ± 0.2, atenolol mean = 1.4 ± 0.1; PSD: saline mean = 1.0 ± 0.1, scopolamine mean = 0.7 ± 0.2, atenolol mean = 1.4 ± 0.1).
mean = 1.5 ± 0.1). These results do not demonstrate a statistically reliable difference in response magnitude across training groups, although autonomic blockade results are similar for CND and PSD subjects (Figure 16). Overall autonomic analyses of these responses indicate a low to moderate validity for the sympathetic and parasympathetic estimates (Figure 16).
Figure 16. Heart period responses to the probe auditory stimulus after vehicle pre-treatment and autonomic blockade, and derived sympathetic and parasympathetic response estimates. **Upper panel:** Heart period responses after vehicle pre-treatment and $\beta_1$-adrenergic blockade (atenolol), muscarinic blockade (scopolamine), or saline. **Lower panels:** Estimates of sympathetic and parasympathetic contributions to the heart period response and corresponding validity intervals. Stimulus onset is indicated by the vertical line, and responses are depicted as a change from pre-stimulus baseline.
Figure 16.
Effects of probe stimuli on blood pressure response. ANOVAs on pressor and depressor responses revealed only a main effect of Training on depressor responses to probe stimuli (Figure 14; $F(1, 10) = 8.71, p < .015$). Similar to the results for extinction trials, depressor responses were larger in PSD subjects than in CND subjects (CND: saline mean = 24.1 ± 9.0, scopolamine mean = 6.6 ± 1.3, atenolol mean = 26.7 ± 5.9; PSD: saline mean = 47.0 ± 7.7, scopolamine mean = 38.9 ± 5.9, atenolol mean = 46.3 ± 16.1).
Discussion

Classical conditioning was used in the present study as a behavioral model of anxiogenesis. Consistent with the hypothesis that PSD controls would exhibit cardiovascular responses similar to FG 7142 pre-treated subjects, stimulus-evoked depressor responses were enhanced in PSD controls, but not in CND subjects. The heart period response to the probe auditory stimulus in PSD subjects did not, however, demonstrate the enhanced FG 7142-mediated cardioacceleratory response. This failure may have arisen from the considerable variability in response magnitude and topography across subjects. The variability of response to the probe stimulus may arise from an underlying coactivational mode of autonomic control similar to that observed in normal animals using a comparable stimulus (Quigley & Berntson, 1990). To examine the possibility of an underlying coactive mode of control, heart period responses to the CS and probe auditory stimulus were depicted on partial autonomic surface maps (Figure 17). Figure 17 shows that the conditioned response to the probe auditory stimuli arose predominantly from parasympathetic activation and withdrawal, with little alteration in the sympathetic component. Fine-grained autonomic surface depictions as used here, can clarify the underlying modes of control. The mode analysis here must remain tentative.
however, because quantitative autonomic analyses indicated only low to moderate validity of the estimates of autonomic contributions to the heart period response.
Figure 17. Partial autonomic surface maps of conditioned and pseudoconditioned response to extinction and tone trials. The axes units are expressed in msec change in heart period. The center data point indicates the pre-stimulus (0,0) starting point for the response, and lines extending from this point depict the time-varying response in 1 sec intervals. Only the first 15 post-stimulus seconds of the response are shown for clarity. Extinction responses in both conditioned and pseudoconditioned subjects are characterized by a reciprocal sympathetic activation and vagal withdrawal. Similarly, the response (without including response recovery to baseline) in pseudoconditioned subjects to the probe stimulus (tone) also is prominently reciprocal sympathetic. Conditioned subjects, however, in response to the probe tone exhibit a prominent parasympathetic activation and withdrawal with little alteration of sympathetic activity.
Extinction

Figure 17.
Investigators have utilized both sympathetic and parasympathetic blockade to elucidate the underlying autonomic origins of classically conditioned and control (e.g. pseudoconditioned or CS-) responses (Cohen & Pitts, 1968; Dykman & Gantt, 1959; Hatton, Buchholz & Fitzgerald, 1981; Iwata & LeDoux, 1988; Kazis, Milligan & Powell, 1973; Schoenfeld, Kadden, Tremont, McCullough, & Steele, 1980; Thompson, Yavorsky & Natelson, 1988; Turkkan & Kadden, 1979). The data reported here revealed a significantly larger cardioacceleratory response to the CS in CND than in control subjects, consistent with the pattern of most cardiovascular conditioning studies (Cohen & Pitts, 1968; Hatton, et al., 1981; Thompson, et al., 1988). Also consistent with the present results, conditioning studies utilizing autonomic blockades have generally revealed a reciprocal mode of control underlying conditioned cardiac responses (see Figures 15 & 17), with the notable exception of the study of Iwata & LeDoux (1988) in which subjects were tested in their homecages. Iwata and LeDoux demonstrated a coactivational mode of cardiac control underlying the response to the CS in CND subjects, in contrast to a reciprocal mode in PSD controls. This notable alteration of the autonomic control of a conditioned response may arise specifically from the testing context under which conditioning occurs. Bouton and colleagues (Bouton & Bolles, 1985; Bouton & King, 1983) have demonstrated effects
of context on the acquisition and maintenance of conditioning where the conditioning context serves as an element of the effective CS. Association of the testing context with the occurrence of aversive stimulation, therefore, can modulate conditioned responding. The homecage testing context of Iwata & LeDoux (1988) likely was minimally associated with shock, and may have shifted autonomic control toward a less reactively labile mode, i.e. coactivation. Conversely, in studies using a novel conditioning context, the context would be expected to accrue considerable association with shock, perhaps with a concomitant shift toward a more reactive mode of control such as reciprocity. The fact that conditioned and pseudoconditioned subjects could not be differentiated on the basis of both cardiac and depressor responses consistent with the effects of FG 7142, may have arisen, in part, from a general, aversive contextual modulation of reactivity in both conditioned and pseudoconditioned subjects.

The present study demonstrates a consistent enhancement of the reactive depressor response in PSD, but not CND subjects, consistent with that in FG 7142 pre-treated subjects. In addition, CND subjects exhibited a prominent uncoupled parasympathetic mode of control. Finally, when shock was predictably imminent (conditioned response to the CS), or unpredictable (pseudoconditioned) subjects exhibited a predominantly reciprocal sympathetic
mode of control. These results support the hypothesis that augmented cardioacceleratory and depressor responses may be useful adjunctive indices of an anxiety state. Importantly, future studies will need to evaluate the specificity of these indicators for anxiety relative to other emotional states. Strong psychophysiological inferences can be drawn when both generalizability and specificity of the psychophysiological concomitants are demonstrated (Cacioppo & Tassinary, 1990).
CHAPTER VII
GENERAL DISCUSSION

The autonomic space model (equation 6) provides a useful conceptual framework in which to delineate general features of autonomic control. The model permits a comprehensive quantitative specification of target organ functional states and reactive responses, and permits a depiction of basal functional loci and time-varying phasic responses. The quantitative methods outlined in Chapter 3, along with the autonomic space model derived in Chapter 2 provide predicted templates of sympathetic and parasympathetic control, and offer validity metrics for the interpretation of autonomic blockades. Valid sympathetic and parasympathetic estimates permit the specification of the autonomic mode of control underlying a tonic chronotropic state or phasic response.

These quantitative methods were applied to analyses of the effects of FG 7142 and aversive conditioning on autonomic and cardiovascular responses to nonsignal and signal stimuli. Data presented in Chapter 5 demonstrate that the BZR partial inverse agonist, FG 7142 enhanced the reactive cardioacceleratory and depressor responses to a
nonsignal auditory stimulus. In the subsequent chapter, it was hypothesized that pseudoconditioning would produce a generalized increase in reactivity similar to that produced by FG 7142. Consistent with the association of a signal stimulus (CS) and shock, the conditioned subjects exhibited an enhanced reactive cardioacceleration, and pressor response to the CS that was not evident in pseudoconditioned controls. Moreover, pseudoconditioned subjects exhibited an enhanced depressor response to both signal (CS) and nonsignal (tone) stimuli, consistent with the augmented depressor response in FG 7142 pre-treated subjects. Also concordant with the effects of FG 7142, an aversive conditioning context increased the error bias in autonomic estimates for both conditioned and pseudoconditioned subjects. The conditioned response to the CS consisted of a reciprocal (sympathetic) mode of control, however, for these subjects, the response to a nonsignal tone evidenced considerable uncoupled parasympathetic control. In contrast, pseudoconditioned subjects showed a predominant reciprocal (sympathetic) mode of control in response to both the CS and the probe tone (prior to the response recovery to baseline). Thus, although the magnitude of the cardiac responses did not differentiate these groups using typical statistical procedures, the autonomic modes of control provided considerable interpretive power. The conclusions about autonomic modes remain tentative, however, as the
validity indices indicated some level of error bias in the autonomic estimates.

The analysis of autonomic effects in FG 7142 pretreated animals suggested a confound of the autonomic blockades with the effects of FG 7142. A reasonable hypothesis for the discrepancies in autonomic estimates suggests that FG 7142 increased central cholinergic outflow as a result of decreasing the effects of GABA on central cholinergic systems. Our laboratory has begun to investigate the possible central mechanisms involved in the FG 7142-induced alteration of autonomic and cardiovascular reactivity. Intracerebro-ventricular administration of carbachol (a muscarinic agonist), results in an enhanced stimulus-induced response to a nonsignal tone that can be blocked by central administration of the muscarinic antagonist, atropine. Further examinations of this hypothesis might involve systemic administration of the relatively cardioselective M2 antagonist, AF-DX 116 to eliminate the potential central alterations of the FG 7142-mediated cardiovascular response by scopolamine methylnitrate (Pitschner, 1991; Pitschner, Schulte, Schlepper, Palm, & Wellstein, 1989). An agent such as AF-DX 116 may also elucidate the mode of autonomic control underlying the FG 7142-mediated response. If these results support the preliminary hypothesis of an FG 7142-induced enhancement of central cholinergic function, it will become
important to determine the specific neuroanatomical substrates underlying this enhanced cholinergic activity.

As noted in Chapter 1, the model of autonomic space is not limited to a single organ system or organism. Future directions may include deriving autonomic space equations and depictions for other species (e.g., dogs for which there exists a considerable literature), or for the development of autonomic control of an organism. For example, an autonomic space derivation for control of heart period in the developing organism would likely encompass shifts in the parameters of the autonomic space equation with changing developmental status. Previous evidence in rats suggests, for instance, that parasympathetic tonic control of the heart becomes evident later in maturation than sympathetic tonic control (Tucker, 1985; Tucker & Johnson, 1984). In addition, intrinsic heart period becomes shorter with increasing age in rats (Tucker, 1985; Tucker & Johnson, 1984). Changes in sympathetic and parasympathetic functional effects and differences in intrinsic function will alter the parameters of the autonomic space equation, necessitating different equations for different age groups. Developmental autonomic space depictions likely will reveal interesting features of the maturation of autonomic control of the heart.

The data presented here demonstrate quantitative methods for assessing the effects of autonomic blockade.
Moreover, these data illustrate the potential organizational principles which can arise from an analysis of autonomic modes that are inapparent from simple analyses of cardiovascular response. Overall, the autonomic space model and accompanying quantitative analyses provide a means for quantifying and evaluating the basic features of autonomic control.
BIBLIOGRAPHY


