THE EFFECT OF TACHYCARDIC IRREGULARITY ON VENTRICULAR REPOLARIZATION DYNAMICS

By

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ABSTRACT

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By the year 2050, it is estimated that 10 million Americans will be living with atrial fibrillation, a fast and chaotically irregular heart rhythm. For years, case reports and clinical studies have shown that patients paradoxically die suddenly from new ventricular arrhythmias occurring soon after the termination of atrial fibrillation, a condition called proarrhythmia. In contrast, patients have a low risk of these arrhythmias while they experience atrial fibrillation. This dichotomy of risk is not seen in patients with regular tachycardias. This dissertation includes the hypothesis that the irregularity of the ventricular response observed during atrial fibrillation alters repolarization in a way that could confer protection from developing repolarization-related arrhythmias. Because many patients with atrial fibrillation are treated with drugs that block I_{Kr}, such as dofetilide, and nearly all forms of proarrhythmic death are associated with abnormal repolarization caused by blocking I_{Kr}, an additional hypothesis is that repolarization may be further altered with dofetilide treatment. To test these hypotheses, domestic pigs were anesthetized and paced in the right atrium of the heart with a random sequence to mimic the irregular tachycardia of atrial fibrillation. Controls were designed to account for the heart rate variation of atrial fibrillation. Since QT intervals vary with heart rate, formulas have been developed to correct the QT interval for changing rates. These correction formulas were tested and found to be

unreliable for group QT correction, therefore in the dofetilide arm of these studies, constant rate pacing at 150 beats/min was used for baseline comparisons. After fixed, sinusoidal, and random tachypacing treatments, these studies found that the QT interval and T_pT_e segment did not change, while the beat-to-beat variability index and QT interval recovery varied extensively across individual pigs. Each pig had a unique response, but no pattern could be attributed to the type of tachypacing. Dofetilide, when administered to pigs, has an unusual dual-effect elimination that has not been previously described. The serum concentration of dofetilide, was lower during random tachypacing compared to sinusoidal tachypacing, suggesting that the clinical theory of protection may be due to a pharmacokinetic protective effect during atrial fibrillation. Random and sinusoidal tachypacing in dofetilide treated pigs is associated with an increase in the RT interval just after the termination of tachypacing. This suggests that tachycardia of any type may confer a post-tachycardia vulnerability though irregularity per se did not alter repolarization differently than the control forms of tachypacing.

The efforts and any benefits associated with the information summarized within are dedicated to my parents, whose simple life lessons drove my motivation toward academics.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

AF	Atrial fibrillation
ANOVA	Analysis of variance
ATPase	Enzyme that catalyzes breakdown of adenosine triphosphate
ATXII	Anemone toxin
AUC	Area under the curve
AV	Atrial ventricular
beats/min	Beats per minute
BPM	Beats per minute
BVR	Beat-to-beat variability
Ca^{2+}	Ionized calcium
CL	Cycle length
CO_2	Carbon dioxide
*d*P/*dt*	Derivative of pressure to the derivative of time
E-4031	Experimental class III antiarrhythmic drug
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
EGM	Endocardial electrogram
f	Frequency
FDA	US Food and Drug Administration
hERG	Human Ether-A-Go-Go
HPLC	High pressure liquid chromatography

HR	Heart rate
HRA	High right atrial
Hz	Hertz
IACUC	Institutional Animal Care and Use Committee
I_{CaL}	L-type calcium current
I_{k1}	Inward potassium current rectifier
I_{Kr}	Delayed rectifier outward potassium current
I_{Ks}	Slow-delayed rectifier outward potassium current
I_{Na}	Fast sodium current
I_{NCX}	Sodium-calcium exchanger
I_{to}	Outward potassium current
$I_{to,f}$	Outward potassium current/ fast
$I_{to,s}$	Outward potassium current/ slow
kHz	Kilohertz
Kv	Voltage gated potassium channel
KVLQT1	Potassium channel protein is encoded by the KCNQ1 gene
K_e	Elimination rate Constant
LQT	Long QT
LQT1	Long QT type 1
Fisher's LSD	Least significant difference test
LVe	Left and right ventricular electrogram
LVP	Left ventricle pressure
LVP_{peak}	Peak left ventricle pressure

mcg/kg	Microgram per kilogram
mcg/kg/min	Microgram per kilogram per min
Mg^{2+}	Ionized magnesium
min	Minutes
minK	Can assemble with KvLQT1 to form a slow delayed potassium rectifier channel
ml	Milliliter
ml/kg/hour	Milliliter per kilogram per hour
mM	Millimolar
mm	Millimeter
mmHg	Millimeters of mercury
mRNA	Messenger ribonucleic acid
msec or ms	Milliseconds
mV	Millivolts
NaCl	Sodium chloride
NCX	Sodium-calcium exchange
ng/ml	Nanograms per milliliter
NIH	National Institutes of Health (United States)
nm	Nanometer
PK	Pharmacokinetic
PKA	Protein kinase A
PKC	Protein kinase C
PR (interval)	ECG time period from the start of the p-wave and ending at the start of the QRS complex

PreFP	Pre-fixed pacing
PreRP	Pre-random pacing
PreSP	Pre-sine wave pacing
PVT	Polymorphic ventricular tachycardia
QRS	Q-wave R-wave S-wave
QTc	Corrected QT interval
QTI	QT interval
QTvi	QT variability
RRA	Repolarization-related arrhythmia
R-R intervals	R-wave to R-wave of the ECG
RSQR	R^2
RTc	RT correction
RTI	RT interval
RV	Right ventricular
RVe	Right ventricular electrogram
RTvi	RT variability
SCD	Sudden cardiac death
SEM	Standard error of the mean
Stim	Pacing
t_0	Initial time
tau	Time constant
T_{end}	End of the T-Wave
Torsades	Torsades de Pointes

T_pT_e	Peak of the T-wave to the end of the T-wave
µl	Microliter
V1	Electrocardiographic lead corresponding to leave V1
VW	Vaughan-Williams antiarrhythmic classification

CHAPTER ONE - INTRODUCTION

OVERVIEW

Atrial fibrillation (AF) is the most frequent arrhythmia encountered in clinical practice today. Additional clinical trials have shown that ventricular heart rate control provides equivalent benefit to rhythm conversion into sinus rhythm. As a result, more people than ever are living with atrial fibrillation and an irregular ventricular response. For many years, case reports and clinical studies have pointed out that patients paradoxically had sudden death from new ventricular arrhythmias occurring soon after the termination of AF. Many of these arrhythmias are thought to be associated with altered repolarization, or “repolarization related arrhythmias” (RRAs). RRAs such as Torsade de Pointe are associated with prolongation of the action potential, often due to abnormalities in ionic currents. There is a correlation between the magnitude of this repolarization delay and the risk for RRA. Some of the drugs used to convert the rhythm of AF to sinus rhythm, and to facilitate electrical or ablative rhythm conversion impair repolarization currents and prolong the QT interval of the electrocardiogram; a surrogate for action potential duration.

Patients with AF have a lower incidence of repolarization-related arrhythmia (RRA) compared to patients who experience RRAs soon after the termination of atrial

fibrillation. This occurs whether the termination mechanism is drug or a combination of drug and radiofrequency ablation. The same phenomenon is not seen in other regular forms of supraventricular tachycardia. When controlled for similar ventricular rates and medications, patients who remained in AF had lower rates of ventricular arrhythmias than AF patients converted back to sinus rhythm. This suggests that the irregularity and not the rate of the tachycardia may be involved in protection from repolarization-related arrhythmia. It is hypothesized that the irregularity of the ventricular response observed during atrial fibrillation alters repolarization in a manner that could confer protection for the development of repolarization-related arrhythmias and/or that conversion from the AF state to a normal regular rhythm is associated with increased RRA risk. Since many patients are converted from AF to sinus rhythm using (in part) I_{Kr} blocking medications, delayed rectifier antagonism may be a requirement for unmasking the proarrhythmic potential following the termination of atrial fibrillation.

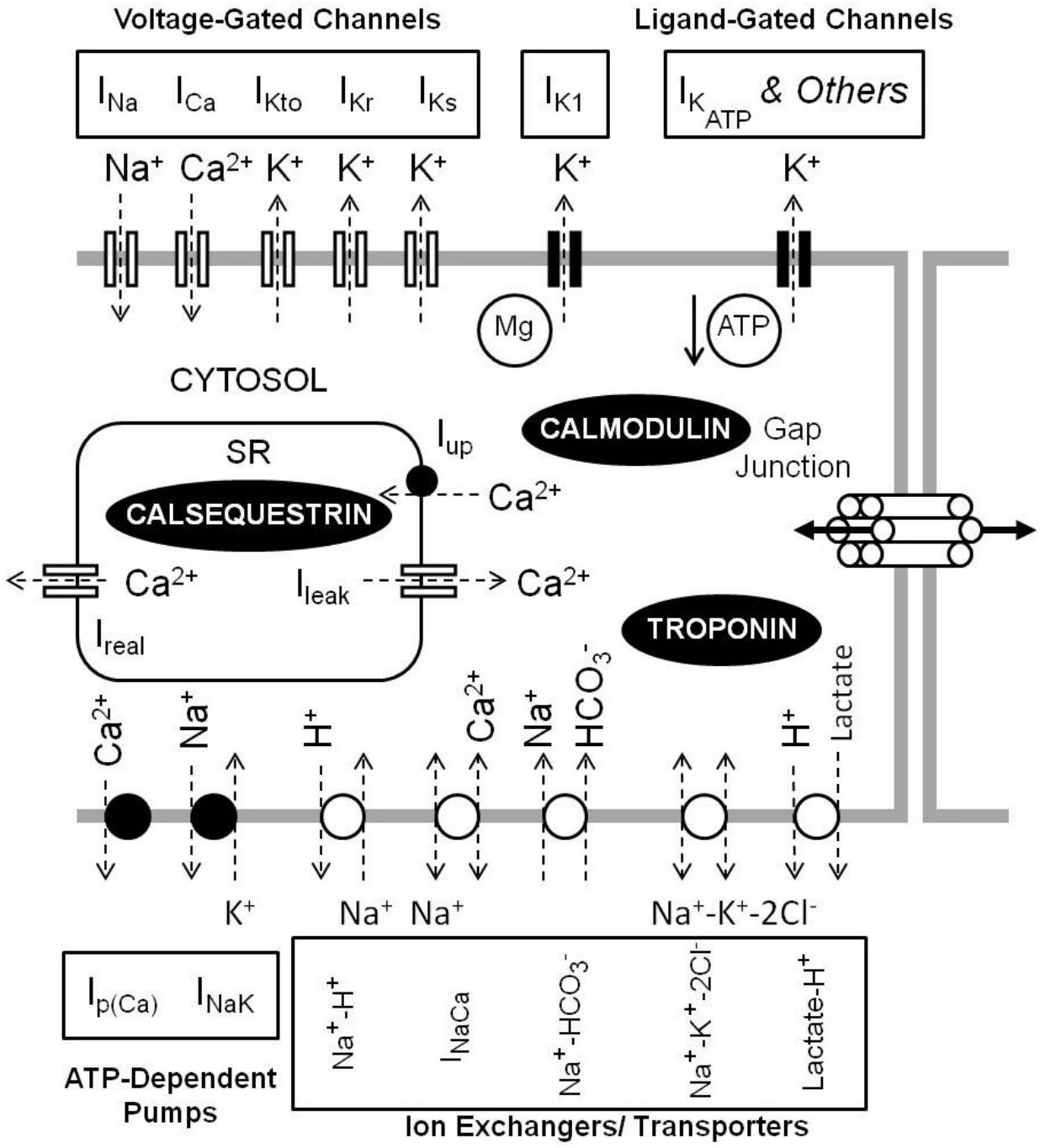
If the irregularity of atrial fibrillation is related to its apparent protective effect, it might occur through a modifying effect of irregularity on repolarization properties of the heart. There remains a paucity of information elucidating the mechanisms responsible for atrial fibrillation induced repolarization modification. The irregularly of atrial fibrillation is a defining characteristic, making it a prime candidate for study. A synthesis of the recent evidence points to the need for a more complete investigation of how irregularity influences cardiac repolarization and the development of repolarization-related arrhythmia. Controlled studies in animal models are needed to more fully understand

these mechanisms. This dissertation reports on the acute effects of transient irregular tachypacing on ventricular repolarization.

INTRODUCTION TO CARDAC ELECTRICAL PROPERTIES

Impulse formation and propagation within the heart is determined intrinsically by the electrical characteristics of the cardiac cell membrane, ion channel function, intracellular and tissue factors. Membrane-bound proteins carry current, in the form of transported ions and respond to cellular signaling molecules. There is a complex interaction between these transmembrane proteins (Figure 1.0). Excitatory currents are carried by the voltage-dependent sodium and calcium channels and are transferred from cell to cell through gap junctions. These junctions are one factor responsible for determining the speed of impulse propagation, while the type and distribution of potassium channels (and to a lesser extent chloride channels) determine the time course of repolarization.

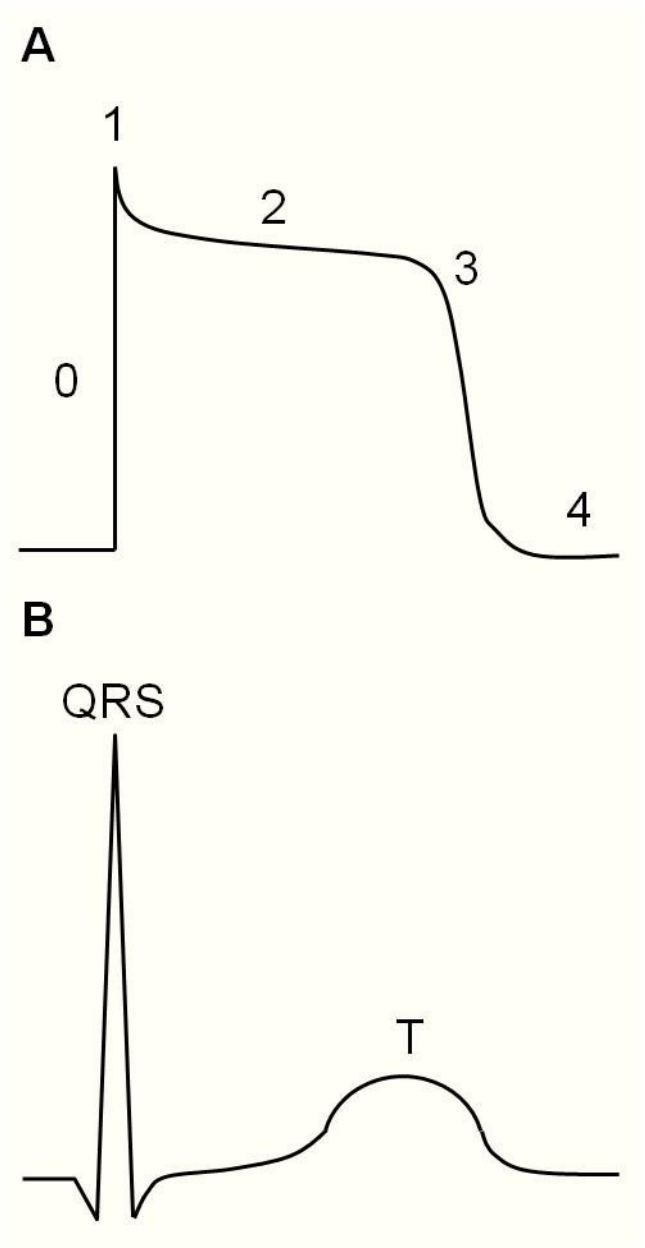
Figure 1.0 Diagrammatic representation of an excitable myocyte with membrane bound channels, pumps and ion exchangers/ transporters. Collectively these contribute to the electrical properties of an action potential. Important ligands that alter the conductance of the outward K^+ currents are depicted in circles adjacent to the protein. The sarcoplasmic reticulum (SR) contains ATP-dependent Ca^{2+}-pumps and Ca^{2+}-release channels. Gap junctions function to electrically couple adjacent cells.



Currents across the cell membrane result in a time-dependent change in voltage and are graphically represented as the action potential (Figure 1.1A). The activation of the contractile components results from the rise in intracellular calcium brought as a result of the voltage change occurring throughout the phases of the action potential (i.e. 0-4). Small fluxes in calcium are augmented by the so-called calcium induced calcium release from the sarcoplasmic reticulum though the interaction with the cardiac ryanodine receptor. The action potential represents the time-varying voltage pattern for an individual cell, or groups of cells. The characteristics for a given group of cells are determined by the cells gene and protein expression. Within the heart there is a diversity of channels with particular properties. The action potential morphology varies among heart regions and when perturbed by extracardiac stressors. Regional differences are also determined by the spatial heterogeneity of channel protein expression. The molecular expression of membrane proteins is a dynamic process with a relatively short time constant that can be altered by stressors (such as disease) and contributing to differences in channel type and density: an idea typically referred to as electrical remodeling.

Action potentials are not typically recorded in clinical medicine. This is in part due to the difficulty and potential complications brought about by the inherent invasiveness of their acquisition. As an alternative, the electrocardiogram (ECG) is used to measure electrical events through the recording and augmenting of electrical signals on the surface of the body. The ECG represents electrical events for the entire heart (Figure 1.1B).

Figure 1.1 Representation of an action potential (A) and its temporal relationship to the electrocardiogram (B).



REPOLARIZATION-RELATED ARRHYTHMIAS

The term polymorphic ventricular tachycardia (PVT) resulted from a series of syndromes described in the 1950's and 60's that first described sudden death associated with QT interval prolongation (Jervella & Lange-Nielsen, 1957; Ward, 1964). The term Torsades de Pointes or simply Torsades was popularized soon after to describe a specific type of PVT that arose from QT interval prolongation and slow heart rates (Dessertenne, 1966). It was found that drugs could produce a similar syndrome of QT interval prolongation and PVT (Selzer & Wray, 1964; Redleaf & Lerner, 1968).

Cardiovascular (antiarrythmics) and non-cardiovacular drugs can both cause QT interval prolongation. Terfenadine, a drug introduced in the United States in 1985, was reported to induce PVT in the late 1980's and resulted in its withdrawal by the FDA (Monahan et al., 1990). Terfenadine blocks the delayed rectifier current, I_{Kr} (Honig et al., 1992; Honig et al., 1993; Woosley et al., 1993]) resulting in QT interval prolongation and proarrhythmia. Delayed rectifier current antagonism is the mechanism responsible for most forms of drug-induced PVT (Roden & Viswanathan, 2005). It is also the underlying basis for the term repolarization-related arrhythmias (RRA) used in this dissertation. As a consequence of this and other evidence, regulatory agencies such as the FDA consider the risk of RRA to be a critical element of drug safety. Prolonged ventricular repolarization has been used as a surrogate marker to suggest a heightened potential for developing RRA. Essentially all compounds in development now must be tested for their QT interval prolonging propensity (Roden et al., 2007). Drug induced

prolongation of 6 milliseconds or more can flag a compound for more regulatory scrutiny (Shah, 2005; USFDA, 2005; Darpo et al., 2006). Though QT interval prolongations have been correlated with increased risk of RRA; the association remains poorly defined. Therefore a comprehensive assessment of repolarization (not just prolongation) is widely considered to be important because a variety of repolarization perturbations can lead to conditions of electrical instability and potentially SCD.

It is known that PVT, specifically Torsades, can degenerate to lethal arrhythmias such as ventricular fibrillation or asystole (Del Rosario et al., 2010). This manifestation of electrical instability and sudden cardiac death are clinically important end-points and a heightened understanding of the causative mechanism is paramount.

ARTRIAL FIBRILLATION

As first defined by William Harvey in 1628, atrial fibrillation is a supraventricular tachyarrhythmia with progressive deteriorating atrial mechanical function (Harvey, 1628). On an electrocardiogram, it is characterized by the replacement of P-waves with oscillations or fibrillatory waves of various morphologies, often with a rapid ventricular response that is classically described as irregularly-irregular. The ventricular rate varies based primarily on the electrophysiology of the AV node. It is modulated by autonomic tone, drug therapy, and structural changes (Fuster et al., 2011). The typical course of atrial fibrillation is a ventricular rate higher than sinus rhythm (Prystowsky, 2008).

Atrial fibrillation is the most frequent arrhythmia encountered in clinical practice today. It is an emerging epidemic that carries with it increased risks of thromboembolic stroke, congestive heart failure, cognitive decline, and premature death. Paroxysmal atrial fibrillation episodes self-terminate. Persistent atrial fibrillation will not stop spontaneously but sinus rhythm may be restored with treatment, and permanent atrial fibrillation occurs when all attempts to restore sinus rhythm have been abandoned. The natural history is for paroxysmal episodes to increase until they become persistent. Therefore, atrial fibrillation is a progressive disease that goes through periods where the episodes of fibrillation can last, for the average patient, for several minutes to several hours (Fuster et al., 2006). The lifetime risk of developing atrial fibrillation for men and women over age 40 is 25% (Lloyd-Jones et al., 2004). Moreover, the number of persons in the United States living with atrial fibrillation is projected to exceed 10 million by 2050 (Miyasaka et al., 2006). The mechanism for induction and perpetuation of atrial fibrillation is variable and complex despite it being the most common arrhythmia encountered in clinical medicine (Richter et al., 2011).

The number of publications relating to the study of atrial fibrillation has increased 8-fold in a 15 year period (1983-1998) and outpaced the literature of other arrhythmias (Prystowsky, 2008). Despite this trend, current therapies remain suboptimal. The advent of both modern drugs that selectively block cardiac ion channels and of advanced interventional techniques provide a modest degree of risk-reduction in mortality and morbidity related to long-term atrial fibrillation (Fuster et al., 2011). Highly effective curative or palliative treatments are, however, still unavailable. Historically, the

treatment of atrial fibrillation was focused on converting the rhythm of atrial fibrillation to sinus rhythm. However, the current treatment consensus is targeted at lowering the ventricular rate to control the symptoms of atrial fibrillation. This was derived from multiple studies summarized in the AFFIRM trial that showed no improvement in the morbidity, and a trend toward lower mortality, among people who remained in atrial fibrillation with a slower ventricular rate versus those converted to sinus rhythm (Fuster et al., 2006; Camm et al., 2007; Camm, 2010; Fuster et al., 2011). This strategy leaves a large population living with an irregular ventricular response. The substantial lifetime risk coupled with a lack of mechanistic evidence, despite a high level of relevant clinical publications and a growing population living with *stable* atrial fibrillation, suggests a need for further mechanistic studies in model organisms.

It seems paradoxical that the restoration of sinus rhythm from atrial fibrillation would increase the risk of arrhythmia. However, within an hour after the conversion of supraventricular tachycardia (with a 76% frequency of atrial fibrillation) to sinus rhythm, studies have suggested that the incidence of developing repolarization-related arrhythmia could be as high as 62% (Minardo et al., 1988; Prystowsky, 1996).

Atrial fibrillation is distinct in part due to its irregular ventricular rhythm. The potential altered risk for RRA, imparted by AF, has not been clinically observed during or following the termination of other supraventricular tachycardias. Fixed-rate supraventricular tachycardias as compared to AF have not been noted to have a decreased incidence of RRA during tachycardia (Martin et al., 2011). Shortly after the

termination of atrial fibrillation, there is an increased incidence of RRA; this has not been observed after other fixed-rate tachycardias. This is true even when controlling for the average ventricular rates during the tachyarrhythmias (Page et al., 2002). In AF, patients with similar ventricular rates who were converted into sinus rhythm had a higher rate of RRA than those who remained in AF (Darbar et al., 2008). Therefore, it is unclear if these findings represent a protective factor operating through AF or a post-conversion vulnerability due to the transition to *normal* sinus rhythm, or both. For either instance, this suggests that the irregularity, and not the rate, of the tachycardia may be involved in modulating the post-conversion vulnerability or protection from RRA. Similarly, if having AF exerts a protective affect by decreasing the risk for the development of RRA, it follows that after the termination of AF; repolarization is restored to a more arrythmogenic state and can be detected.

ANTIARRHYTHMIC DRUGS

Many antiarrhythmic drugs carry a proarrhythmic risk of greater than one percent. Dofetilide, sotalol, and quinidine can produce this cardiotoxic effect by blocking the delayed rectifier current similarly to terfenadine (Roden et al., 1986; Soyka et al., 1990; Coplen et al., 1990; Torp-Pedersen et al., 1999; Darpo, 2007) Speculations regarding the discrepancies between the incidence of RRAs among non-cardiovascular (<1/10,000) and cardiovascular drugs include the individual characteristics of the population treated with these drugs, and the magnitude of the QT interval prolongation (Roden et al., 2007). It is understood that certain populations are more vulnerable:

females and patients with pre-existing sarcolemal ion channel dysfunctions ("channelopathies"), hypokalemia, bradycardia, and/or heart block (Roden, 1998). Despite these known risks enhancers, some patients have high risks for reasons that are still unclear. These population characteristics are often not accounted for in human studies examining the relationship of AF to repolarization, refractoriness, or RRAs, suggesting that animal models may be needed to more completely understand the individual factors that modify repolarization and refractoriness (Darpo, 2007).

Many antiarrhythmics modify the ventricular rate, a phenomenon associated with a modification of repolarization and refractoriness. In animals and humans, low ventricular heart rates are associated with an increased likelihood for the development of RRAs (Brandt & Win-Kuang, 1995). It has been suggested that the incidence of RRA would be negligible above a threshold ventricular rate defined as more than 70 beats/min (Pinski et al., 2002). The QT interval is rate-dependant and prolongs at low heart rates; therefore it is possible that the QT interval prolongation observed at low heart rates (or because of a combination of factors or epiphenomenon) imposes arrhythmia vulnerability.

Women have a higher risk than men for developing RRAs in response to compounds that prolong cardiac repolarization. At birth, the QT interval adjusted for heart rate is similar between men and women (Stramba-Badiale, 1995). During and after puberty, there is a shortening of the QT interval corresponding to a rise in testosterone in males, which is thought to account for the heightened risk of RRA among females (Rautaharju,

1992; James et al., 2007). Further studies show that estrogen like hormones decrease I_{Kr} and testosterone hormones increase I_{Kr} expression (Kurokawa, 2008). Sex hormones also alter the binding properties of drugs to I_{Kr} ion channels (James & Hancox, 2003; Kurokawa, 2008). Recent studies have supported the role of testosterone in a reduced risk of RRAs in males (Jonsson, 2010): the activation of I_{Kr} channels via testosterone binding to androgen receptors (Ridley, 2008). However, despite extensive studies, the exact biological basis for these observations has not been determined (Kurokawa, 2008).

In a recent study, Boulet described a loss-of-function mutation in the KCNQ1 gene that encodes for the I_{Ks} channel in a patient who had episodes of RRA and a normal QT interval suggesting that repolarization channelopathies may promote RRA but not manifest repolarization delay (Boulet, 2006). This type of “concealed long QT syndrome” suggests the need for alternative, clinically practical diagnostic measures of repolarization that assess the risk for repolarization impairment with high fidelity (Varro & Papp, 2006). Individuals with genetic abnormalities relating to abnormal repolarization ion channels may be more at risk for RRA that those with a normal genetic background.

Hypokalemia and, to a lesser extent, hyperkalemia have been associated with the development or RRA. Low extracellular potassium increases I_{k1} and I_{Ks} and reduces I_{Kr} (Yang et al., 1997). These alterations favor QT interval lengthening (Sanguinetti &

Jurkiewicz, 1992). I_{Kr} blocking drugs when administered to subjects with a potassium deficiency have been implicated in depressing the repolarizing current (Yang & Roden, 1996). However, this finding seems paradoxical when considering the increase risk for RRA with high potassium, and therefore recent findings have been questioned (Limberis, 2006).

REPOLARIZATION IMPAIRMENT

The concept of "repolarization reserve" has emerged in the last 15 years as a theory (Roden, 1998, 2006, 2008) to explain impairment of repolarization based on multiple factors responsible for risk or protection from RRAs. This concept has been validated experimentally (Iost, 1999; Varro, 2000, Lengyel, 2001, Jost, 2005) and has been used to explain RRAs from complex pharmacology often complicated by systemic disease. This principle has been attributed to known conditions that modify the risk for the development of RRAs: drug effects, ion channels, genetic determinates, and certain diseases. The theory of repolarization reserve is explained by Roden as:

> *"The concept of "repolarization research," the idea is that the complexity of repolarization includes some redundancy. As a consequence, loss of 1 component (such as I_{Kr}) ordinarily will not lead to failure of repolarization (i.e. marked QT prolongation); as a corollary, individuals with subclinical*

lesions in other components of the system, say I_{Ks} or calcium current, may display no QT change until I_{Kr} block is superimposed." (Roden, 2008)

Recent studies have indicated that repolarization reserved is a dynamically changing property of cardiac muscle. I_{Kr} antagonists, such as dofetilide when incubated with dog ventricular myocytes elicited shorter action potential duration and blunted dofetilide induced prolongation (Xiao et al., 2008b). Enhancement of I_{Ks} was noted in myocytes exposed to chronic dofetilide compared to untreated cell during patch-clamp experiments. The MinK and KvLQT1 protein levels were increased; however, the mRNA fold change was unaltered, suggesting post-translational regulation. It was also shown that microRNA was attenuated in cells treated with dofetilide (Luo et al., 2007). Based on these findings, chronic reduction of I_{Kr} leads to upregulation of I_{Ks}, representing an example of feedback of ion channel function.

Repolarization impairments can be difficult to measure and quantify. In the laboratory, repolarization reserve can be measured by drugs that enhance repolarization (e.g. veratridine or ATXII). Treatment would be expected to produce action potential shortening that opposes the potassium channel current. The observed limited action potential duration predicts, and is correlated to, strong repolarization reserved. I_{Kr} inhibition may also be used for assessing impairment of repolarization and may be preferable because most drugs that have liability for RRAs, inhibit this current. The

evoked repolarization lengthening mainly assesses currents other than I_{Kr} where I_{Kr} functioning is preserved (Volders et al., 1999).

Traditional QT interval or QTc measurements from electrocardiograms are likely not sufficient to fully evaluate repolarization. The addition of other parameters such as T_{peak} to T_{end}, QT variability index (Thomsen et al., 2004), or short term QT variability (Berger, 2003) may have value in higher risk patients in the clinic for RRAs. Pharmacologic tests to evaluate repolarization are an additional option; however, these types of clinical studies raise ethical concerns in humans related to their risk/benefit profile (Kilborn et al., 2000).

MOLECULAR DETERMINATES OF REPOLARIZATION

Heart rate, through impulse formation and repolarization, is determined by the electrophysiology of the cardiac myocytes functioning as individual cells and complexed with other cells as tissues and organs (figure 1.0). Sarcolemmal membrane proteins act to transport ions and modulate mechanical stress while responding to internal and external signals. Protein channels of the myocyte membrane respond to voltage or ligands that modify the opening, closing, inactivation, and signal processing of the cell. Specific potassium and calcium channels determine the kinetics of repolarization and refractoriness. These channels reflect a complex relationship that impacts arrythmogenesis and are described below.

During the short period of time when activation and inactivation coincide, a sodium current called the Na^+ window current can be recorded (Attwell et al., 1979). This fast sodium current (I_{Na}) is largely inactivated within a few milliseconds; however, the sodium current does not disappear completely during the portion of the action potential representing repolarization. During the plateau phase, a persistent sodium inward current, opposing potassium mediated repolarization and delaying complete repolarization (Carmeliet, 1987). Heart failure, long QT syndrome type 3, as well as the administration of veratrine or ATXII increase the sodium mediated repolarization prolongation (Schwartz et al., 1995). The functional change is a decrease in repolarization reserve, or increase in repolarization prolongation, but with a greater increase of inward current; therefore, QT interval prolongation may occur.

The calcium current is also involved in repolarization, but the extent of its involvement is not fully understood and seems to be highly complex. The L-type calcium current (I_{CaL}) exhibits slow inactivation and a tight dependence on cytosolic calcium concentrations. Throughout a period of depolarization and repolarization, calcium concentrations cycle dramatically, regulated by many factors including the sarcoplasmic/endoplasmic reticulum calcium ATPase, phospholamban, ryanodine receptor, calmodulin, as well as protein kinase A and C (Bers, 2001). The impact from alterations in these factors is difficult to predict accurately and remains one of the most elusive targets for action potential modeling. An examination of increased inward current, including both sodium and calcium, reveals a shift in the positive direction of the plateau phase imparting an

enhancement in activating potassium current, an outward current. This alteration, depending on the function of other currents, would shorten repolarization.

I_{NCX} (NCX) restores low concentration of intracellular calcium during diastole (Blaustein & Lederer, 1999). As the membrane potential is changing throughout the cardiac cycle, three sodium ions are exchanged for two calcium ions in a cyclic electrogenic mechanism that is dependent on intracellular calcium concentrations. Just prior to depolarization, the intracellular calcium concentration is low, the membrane potential is negative, and the NCX is outward. During the plateau phase, early/late repolarization and diastole, the NCX is inward. Under conditions of calcium overload, NCX may act as a trigger for early/late afterdepolarizations that can contribute to RRAs (Bers et al., 2002). The NCX is pathologically upregulated during heart failure (Schillinger et al., 2000). A full understanding of this current is lacking due mainly to the absence of specific antagonists for the NCX. Bers found that NCX is functionally expressed differently throughout several regions of the ventricles, resulting in altered transmural dispersion of repolarization (Xiong et al., 2005). NCX abnormalities in combination with pathological heart failure, decreased repolarization reserve, and altered dispersion, can augment the risk for RRAs.

Calcium cycling has regulatory function on a diverse number of ion channels in the heart. Sodium channels are regulated by calmodulin (Tan et al., 2002) as well as their short-term density distribution (Casini et al., 2009). Rapid pacing, as a model to induce a tachycardiomyopathy in a dog, downregulates I_{t0} via the calcium-calmodulin

dependent protein kinase II and calcineurin/NFAT system (Xiao et al., 2008). Calmodulin, along with calcium and calmodulin-dependant protein kinase II, has not only been shown to regulate I_{t0} (Tessier et al., 1999; Sergeant et al., 2005), but also to correct channel assembly and gating properties of the delayed rectifier current (Shamgar et al., 2006; Ghosh et al., 2006). Calcium was also shown to have a role in regulating the delayed rectifier current in guinea pigs (Tohse et al., 1987) by the inhibition of the hERG channel (Schönherr et al., 2000). Calmodulin-dependant protein kinase II has demonstrated effects on the properties of I_{t0} and I_{k1} in cardiac disease (Nerbonne, 2011). The science behind beat-to-beat variability of repolarization is related to calcium cycling in the chronic AV-block dog model (Oros et al., 2008). Cardiac repolarization T-wave alternans is associated with alterations of calcium cycling (Pruvot et al., 2004). These alterations in calcium handling play a clear role in repolarization.

The inward rectifier potassium current (I_{k1}) operates as an inward rectifier though Kir 2.1, 2.2 and 2.3. The I_{k1} decreases as membrane potentials approach zero and increase as potential become more negative than -30 mV. Matsuda has shown that magnesium (Mg^{2+}) and amines from intracellular sources modulate electrogenic properties (Matsuda et al., 1987). With increasing membrane potentials the Mg^{2+} or amine related block is reduced and I_{k1} increases. Diastole favors channel opening and opposes depolarizations from calcium overload-related delayed afterdepolarization or enhanced automaticity. Dysregulation of these channels favors the triggering of

arrhythmias by permitting the threshold for extrasystoles to be met and propagated. A smaller I_{Kr} increases the total repolarization and heterogeneity; however, this concentration is highly variable between species. Indeed, dogs, guinea pigs, and rabbits have a strong I_{Kr} contribution to repolarization (Varro et al, 1993), while humans have a weak I_{Kr} contribution (Jost et al., 2008). This suggests that these channels play a small yet important role in human proarrhythmia and in combination with other channelopathies, alter the risk for RRAs. Andersen-Tawil-syndrome (long QT type 7), a genetic disorder with a mutation in Kir 2.1, has a high risk for RRA but only a slight QT interval prolongation (Zhang et al., 2005). Modeling experiments have supported that I_{k1} is important for repolarization homeostasis during late repolarization and diastole (Ishihara et al., 2009).

The transient outward potassium current (I_{t0}) has slow ($I_{t0,s}$) and fast ($I_{t0,f}$) components with channels consisting of Kv1.4 and Kv4.2/3, respectively. I_{to} is a large current, at membrane potentials greater than -20 mV, with rapid kinetics for activating (<2 sec) and inactivating (5-10 sec) outward currents (Patel & Cambell, 2005). Although I_{to} largely contributes to phase 1 depolarization, the role it plays in altering the action potential duration is limited. Indeed, this current can affect the amplitude of the resultant plateau phase, thus playing a role in activation, inactivation and deactivation channel kinetics. I_{to} has an additional slower phase of depolarization that is responsible for directly maintaining the plateau of the action potential. The density of these channels varies

between the subendocardium, midmyocardium, and subepicardium. This may contribute to modification of the transmural heterogeneity and the risk for RRA (Litovsky & Antzelevitch, 1988; Antzelevitch & Fish, 2001). Humans and dogs carry the dominant alpha subunit protein (Kv4.3), while rabbits carry the Kv1.4 (Fulop et al., 2006; Wang et al., 1999). This indicates I_{to} is species dependant. In humans, I_{t0} alone does not lead to dramatic repolarization changes but may if complexed with other channel dysfunctions.

The slow-delayed rectifier outward potassium current (I_{Ks}) activates slowly (0.5-1 sec) and deactivates rapidly (100-200 msec) at negative potentials throughout the late plateau phase of the action potential (Jost et al., 2007). It is comprised of KvLQT1, MinK and MIRP protein subunits. Due to the slow kinetics and low amplitude signal (<20 mV), under normal conditions, I_{Ks} has little influence on the absolute duration of repolarization (Varro et al., 2000; Jost et al., 2005). According to Carmeliet, under the influence of a higher membrane voltage and action potential lengthening, I_{Ks} acts as a reserve for additional channels to augment repolarization, and thus is a main contributor toward repolarization reserve by opposing excessive action potential lengthening (Carmeliet, 2006). During sympathetic outflow, I_{Ks} activation via PKA (in addition to its normal activation by PKC), increases the I_{Ks} amplitude and density of I_{Ks} channels, while decreasing the membrane potential (Yazawa & Kameyama, 1990; Walsh & Kass, 1991). Sympathetic activation also enhances L-type calcium release, shifting membrane voltages in a positive direction. The I_{Ks} may act as a negative *feedback*

element, limiting the extent to which repolarization can be prolonged during high sympathetic states (Han et al., 2001; Volders et al., 2003). An example is acquired or congenital long QT 1 (LQT1) syndrome, where an I_{Ks} blocking drug or a loss-of-function mutation, with concomitant sympathetic stimulation, leaves the $I_{CA,L}$ unopposed, resulting in repolarization prolongation leading to a heightened risk for RRAs (Johnson, 2010). Altered I_{Ks} functioning has also been associated with increases in transmural dispersion of repolarization as demonstrated by the establishment of a link between increased differences in regional repolarization and the heightened risk for RRAs (Akar et al., 2002).

The delayed rectifier outward potassium current (I_{Kr}) activates rapidly (<40 msec) at membrane potentials greater than -30 mV. I_{Kr} channels are largely closed during the plateau phase of the action potential, but open when the membrane potentials approach zero. The kinetics of recovery are quick, enabling each channel to cycle during a single repolarization interval. Opposite to what one might predict, increased extracellular potassium concentrations enhance, while decreasing potassium concentrations depress I_{Kr} (Sanguinetti & Jurkiewicz, 1992; Yang et al., 1997). Low potassium concentrations accentuate internalization and degradation of the channel in rabbit hearts and in human cell lines (Guo et al., 2009). It has been suggested that I_{Kr} may play a role in positive feedback by contributing toward repolarization lengthening when repolarization is already prolonged especially do to other factors such as a slow heart rate or

channelopathy (Virag et al., 2009). I_{Kr} plays a similar role to I_{K1}, although I_{K1} is optimal at membrane potentials over a lower range of potentials (Virag et al., 2009). Also, when action potential durations are lengthened, there is a lower repolarization contribution from I_{Kr}/I_{K1}, further weakening the force of repolarization. This supports the concept of heightened vulnerability when depolarizing factors (I_{Na}, $I_{Ca,L}$, NCX) are increased or repolarizing factors (I_{K1}, I_{Ks}, Na/K ATPase) are decreased. A small reduction in I_{Kr} may cause a significant prolongation in repolarization at slow heart rates or have a prolongation effect when combined with other contributors to repolarization prolongation. The delayed rectifier current is thought to be the most important component to repolarization. In most species, including humans, potent inhibition of I_{Kr} has a substantial influence on repolarization lengthening (Varro et al., 2000; Lengyel et al., 2001; Jost et al., 2005). Compensatory mechanisms from outward currents play an important role in the negative feedback on repolarization prolongation, whereby incomplete inhibition of I_{Kr} may not result in detectable repolarization lengthening. I_{Kr} is a major outward current involved in repolarization and because excessive impairment of I_{Kr} leads to RRAs, it represents a primary target for mechanistic studies.

ATRIAL FIBRILLATION, REPOLARIZATION-RELATED ARRHYTHMIA & ANTIARRHYTHMIC DRUGS

It has been clinically noted that repolarization-related arrhythmia is relatively uncommon during atrial fibrillation (Härtel et al., 1970; Arstall et al., 1992; Faber et al., 1994; Hohnloser et al., 1995). However, following the successful conversion of atrial fibrillation to sinus rhythm with antiarrhythmics, it has been observed that the rate of repolarization-related arrhythmias increase in these patients compared to similar patients treated for rate control alone (Stambler et al., 1996; McCray et al., 2006; Darbar & Roden, 2006). A similar finding was shown in a recent systematic study to identify risk factors for RRA in a large group of subjects. One hundred human subjects with atrial fibrillation received an intravenous infusion of almokalant, a drug with specific I_{Kr} antagonist effects, and five out of six patients who developed RRA were in the sinus rhythm group (Houltz et al., 1998). This suggests that a poorly defined characteristic(s) of atrial fibrillation, or the transition from atrial fibrillation to sinus rhythm, is involved in modulating the risk of developing RRAs.

Atrial fibrillation is a major disease that often necessitates the initiation of antiarrhythmic therapy (Darbar & Roden, 2006). Drugs used for the conversion of atrial fibrillation to sinus rhythm during management often prolong ventricular repolarization via hERG channel blockade. Blocking the hERG channel prolongs the I_{Kr} current, the current that is principally responsible for ventricular repolarization and represented globally by the QT interval on the electrocardiogram. In human clinical studies suggesting a protective

effect (from RRA development) imparted from atrial fibrillation or a post-conversion vulnerability following conversion from AF to sinus rhythm, the successful conversion of atrial fibrillation is often improved by the administration of I_{Kr} blockers. Many antiarrhythmics that have antagonistic properties towards the I_{Kr} channel prolong repolarization and also decrease the ventricular rate, a risk factor for the development of RRAs. Numerous studies have demonstrated that I_{Kr} is an important target for understanding the mechanism by which repolarization is linked to AF (Kano et al., 2005). Therefore delayed rectifier antagonism may be a requisite for unmasking proarrhythmic potential following the termination of atrial fibrillation.

The ventricular heart rate is inversely coupled to the QT interval. Atrial fibrillation naturally has a ventricular rate higher than a “normal” sinus rhythm. Recent evidence suggests that the high rate alone may play a protective role from dofetilide induced RRAs (Oosterhoff, 2010). This is in agreement with the evidence that low ventricular rate may increase arrhythmia vulnerability (Pinski et al., 2002). Estimates for the risk of proarrhythmic death following catheter ablative conversion of atrial fibrillation are approximately 5% (Evans, 1991). However, in clinical studies examining the incidence of arrhythmia in patients given similar antiarrhythmic drugs for the treatment of atrial fibrillation, those that remained in atrial fibrillation had a lower incidence of RRA compared to those who were converted to sinus rhythm, despite having similar ventricular rates (Darbar et al., 2008). This suggests that rate alone may be insufficient to account for the potential protection afforded by atrial fibrillation.

The fidelity of the change in the QT interval with ventricular rate is not well coupled. The latency of the QT interval during atrial fibrillation is slow to respond to changes in a beat-to-beat manner. When the QT interval remained relatively steady during atrial fibrillation and following conversion to sinus rhythm, prolongation was observed (Choy, 1999; Darbar et al., 2004). This suggests that a mechanism by which irregular tachypacing alters QT dynamics might require a more complete QT interval assessment. Because the QT interval is evident on the surface ECG recording and is commonly monitored in this class of patients, a more thorough understanding could suggest a convenient method to asses risk in the clinic.

ANIMAL MODEL

Different species have different sets and relative activities of ion channels important to repolarization. (Hashimoto, 2008). Rats, though convenient for study, do not possess the I_{Kr} (rapidly activating delayed rectifier potassium) current, which is vital to the study of RRAs (Regan et al., 2005). Other animals have only weak versions of I_{to} (transient outward potassium current) (Bachmann et al., 2001). Rabbit's possess I_{Kr}, I_{Ks} and I_{to}; however, their functional kinetics differs significantly from that of humans (Janse, 2004). Pigs have repolarization currents similar to humans (I_{Kr}, I_{Ks}, and I_{to}) and are large enough to allow the experimental instrumentation needed for these studies (Salata at el., 1996). Pigs also have heart rate variability that is similar to humans and dissimilar to other potential model organisms (Stubhan et al., 2008).

In vivo animal studies in pigs provide a better understanding of whole animal physiology and mechanisms involved in the pathogenesis of atrial fibrillation. There is a body of arrhythmia literature based on studies in this species, and it is the species closest to humans in which these experiments are technically feasible. This is in part due to their surface area to organ ratio and electrophysiology being similar to those of humans (Rubart et al., 1997; Kates et al., 1984) and in part due to the similarity of the coronary vessels' reactivity and adrenergic receptor distribution between pigs and humans, and dissimilarity to other model systems (Hidaka et al., 1985). Previous studies have used pigs as a model for the study of drug-induced QT interval prolongation (Kano et al., 2005). The porcine model also has the distinct feature of developing congestive heart failure following rapid pacing at rates that approximate those observed in atrial fibrillation that is similar to that which is seen in humans (Kates et al., 1984; Maisel et al., 2003).

OVERALL HYPOTHESIS & SPECIFIC AIMS

The overall hypothesis of this project is that tachycardic irregularity alters ventricular repolarization properties in porcine hearts. Further, this alteration will be consistent with clinical observations for protection, and post-conversion vulnerability, from RRAs in patients with atrial fibrillation. The following specific aims (and hypotheses) are designed to test this overall hypothesis.

First Arm - Specific Aim 1

Evaluate and compare models of tachypacing repolarization during and after fixed and sinusoidal pacing, to random pacing.

Hypothesis

Transient irregular, as compared to fixed and sinusoidal atrial overdrive tachypacing alters ventricular repolarization dynamics.

Second Arm - Specific Aim 2

Characterize the pharmacokinetic and QT effect of dofetilide in domestic pigs.

Third Arm - Specific Aim 3

Evaluate and compare the repolarization effects of dofetilide during and after sinusoidal and random tachypacing.

Hypothesis

I_{Kr} antagonist treatment with dofetilide alters repolarization dynamics in the presence of irregular tachypacing differently than its effects during sinusoidal tachypacing.

CHAPTER TWO - METHODS

OVERVIEW

Several parameters have been used in the literature to quantify repolarization and the likelihood of developing RRAs (Sticherling et al., 2000). The absolute QT interval, corrected QT interval (QTc), variance of the QTc, and the electrocardiographic T wave segment from peak to end (T_pT_e) are typical markers of repolarization. In this research these were measured in domestic pigs before, during, and after periods of tachypacing. Temporal markers of repolarization were also explored, including the gain parameters of the QT interval recovery after tachypacing, and sequential-beat QT interval variability (i.e. Poincare plots). Each was examined immediately following the termination of tachypacing. The QT variance was examined using an index representing the log-ratio between the QT interval variability and the heart rate variability, each normalized to the corresponding mean value. These selections were developed to comprehensively evaluate repolarization during and after tachypacing in *normal* and dofetilide treated pigs. The evaluation of animals given dofetilide was limited to an assessment of hemodynamic parameters, the QT interval and T_pT_e duration.

ANIMALS

All protocols involving animals were approved by the Michigan State University Institutional Animal Care and Use Committee (IACUC); a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International that conforms to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Female farm pigs weighing 35 ($\pm$4) kg were used in most experiments. The time control animals to test the final specific aim of this project included two males in the cohort. In animals given dofetilide, pigs weighing 46 ($\pm$6) kg were used due to animal availability. These animals were acquired from the Michigan State University Swine Teaching and Research Facility and were approximately 3-4 months old and not sexually mature. After arrival, pigs were acclimatized for at least one day under controlled temperature and humidity conditions with alternate 12 hour light-dark cycles. Pigs were monitored daily and offered water ad libitum and food twice per day. The animals enrolled in these studies were checked regularly by licensed veterinarians, veterinary technicians and staff of the vivarium of Michigan State University.

All procedures were performed in fully anesthetized pigs using approved anesthetics for induction and maintenance. In pigs, a lack of response to mild noxious stimuli and muscle tone was used to assess the initial state of anesthesia. During the study, appropriate depth of anesthesia was monitored by the stability of heart rate, temperature, respiratory rate and arterial pressure. Following surgery, all animals were

euthanized by administration of an overdose of sodium pentobarbital (100mg/kg). This method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association (2013 edition).

Sedatives or additional anesthesia adjuvants, utilized to facilitate induction of anesthesia, can alter ion channel function. Therefore, such premedications were not used because *normal* electrophysiology, an important factor in this study, could be altered. For induction of anesthesia, pigs were exposed to isofluorane (5%) using a mask, brought to a suitable plane of anesthesia, for subsequent endotracheal intubation. Anesthesia was maintained using an inhaled isoflurane/oxygen mixture (2.75$\pm$0.5%). Animals were placed on a ventilator with a tidal volume (450$\pm$100ml) and rate (19$\pm$3breaths/min) adjusted to maintain a near normal end tidal pCO2 (Datascope Corp, Paramus, NJ) of 35-45 Torr. A 22-gauge catheter-over needle through the ear vein was utilized to obtain peripheral intravenous access. Intravenous isotonic fluid (Lactated Ringers) was given as a maintenance infusion (2.2ml/kg/hour) to account for sensible and insensible fluid loss.

The neck's vessels were exposed via a minor surgical approach. The right external jugular vein and carotid artery were isolated. The artery was cannulated with a 10F double lumen sheath and the vein cannulated with a 14F triple lumen catheter. These venous ports were used for the insertion of the high right atrial (HRA), right ventricular electrogram (RVe) and right ventricular (RV) Millar pressure catheter. Left ventricular pressures were acquired with a Millar solid state pressure transducer catheter (Millar

Instruments, Houston, TX), advanced through the carotid artery, amplified (Grass Instruments, Warwick, RI) and collected at a sample rate of 1 kHz. The left (LVe) and right (RVe) ventricular electrograms were acquired using a dacron electrophysiology catheter (St. Jude Medical, Minneapolis, MN) advanced into the right and left ventricular apex. The femoral vessels were accessed percutaneously to place a 5F and 7F size single lumen catheter into the artery and vein, respectively. The femoral vein was used for blood collection and the reinfusion of isotonic normal saline (0.9% NaCl) for volume replenishment. The femoral artery was used for peripheral cardiac blood pressure recording using a solid-state external pressure transducer calibrated to mercury. When adequate access to the femoral artery was not possible, access to a second carotid artery was substituted.

Standard surface electrocardiographic leads were collected, including lead V1. All catheter positions were confirmed with fluoroscopy and the voltage for atrial pacing stimulation (SD9 – Grass Produce Group, W Warwick, RI) was established based on doubling the pacing threshold.

HIGH RIGHT ATRIAL TACHYPACING

Central to the first arm of studies in this dissertation is high right atrial pacing which involves fixed, sinusoidal, and random pacing (Figure 2.0). These three types of pacing had the same duration and same average heart rate. Sinusoidal and random pacing had the same variance ($\pm$1.2%). The fixed rate pacing is performed at a constant cycle

length that is equal to the average for sinusoidal and random pacing. The "random" pacing type was a uniformly distributed pseudorandom pattern bounded by the peak and nadir of the sinusoidal R-R intervals. The sinusoidal pattern of pacing is based on an average heart rate, high enough so that the nadir of the wavelength is higher than the animals' natural heart rate. Otherwise, extra beats could occur that could complicate data interpretation. The range of heart rate values was selected based on those commonly encountered during paroxysmal atrial fibrillation. The frequency of the sinusoidal pattern was selected based on the respiratory frequency in a relaxed subject (0.25 Hz)(Stein et al., 1994; Frey et al., 1995). The formula that relates to the sine wave frequency (*f*) in Hertz is:

$$f = \frac{1}{i * \#beats}$$

where " " refers to the average cycle length interval in seconds, and the "*#beats,"* is the number of beats per cycle. A main feature for sinusoidal pacing is that the inflection points and zero crossing (i.e. mean HR crossing) points were forced elements of the pacing sequence. The minimal number of inflection points per cycle is four, and additional points were interspersed between these points based on this equation:

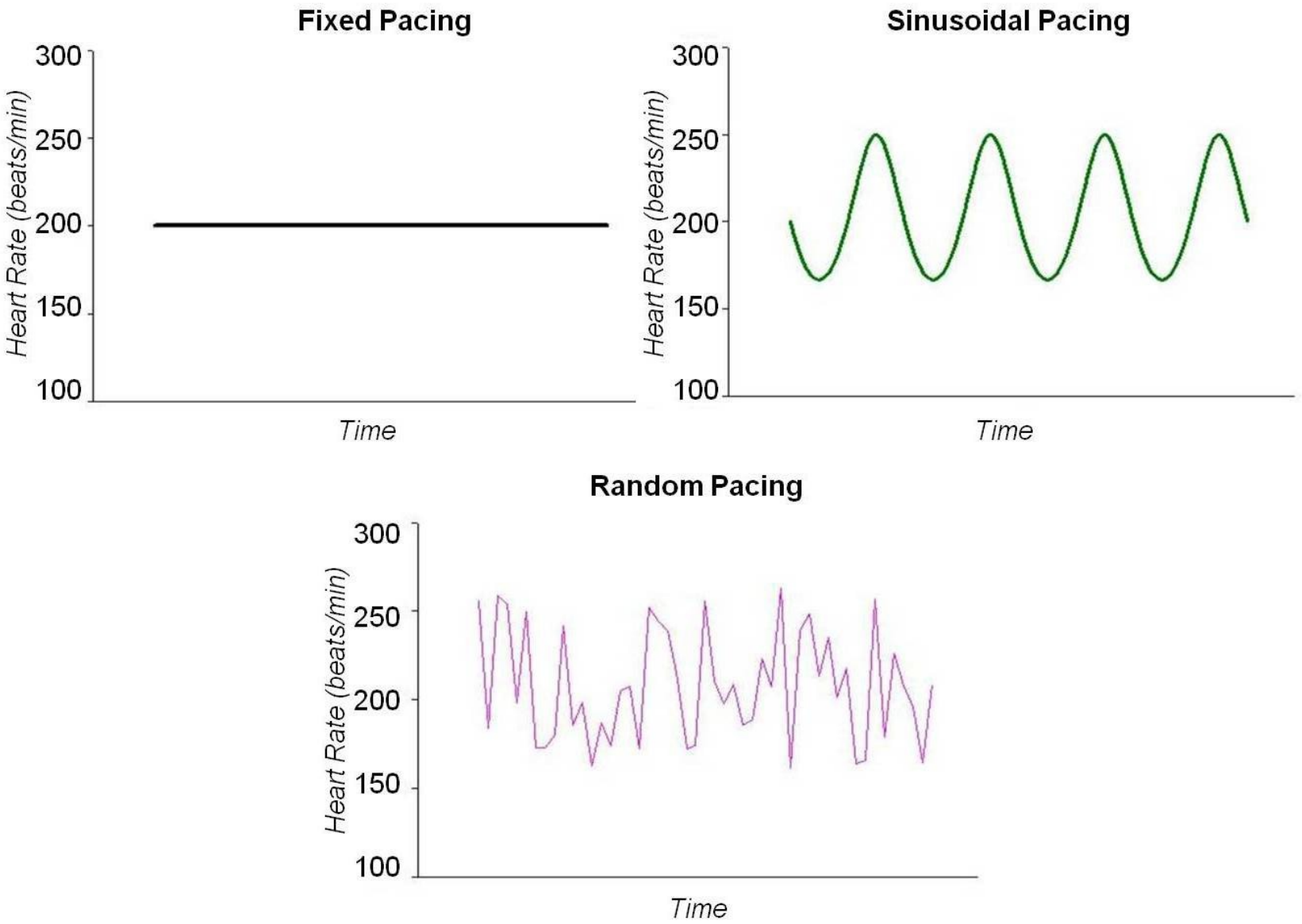
$$\# \, beats = 4 + x(4)$$

where "x" refers to the number of interspersed beats that are zero or positive integers. The more beats per cycle, the more closely this pacing sequence mimics a true sine wave, and the lower the frequency for any given average cycle length.

Figure 2.0 Representation of high right atrial pacing paradigms showing fixed (black), sinusoidal (green) and random (pink) tachypacing as a continuous independent variable. The average heart rate is 200 beats/min and range is ~60 beats/min (for sinusoidal and random tachypacing).

[For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this dissertation.]

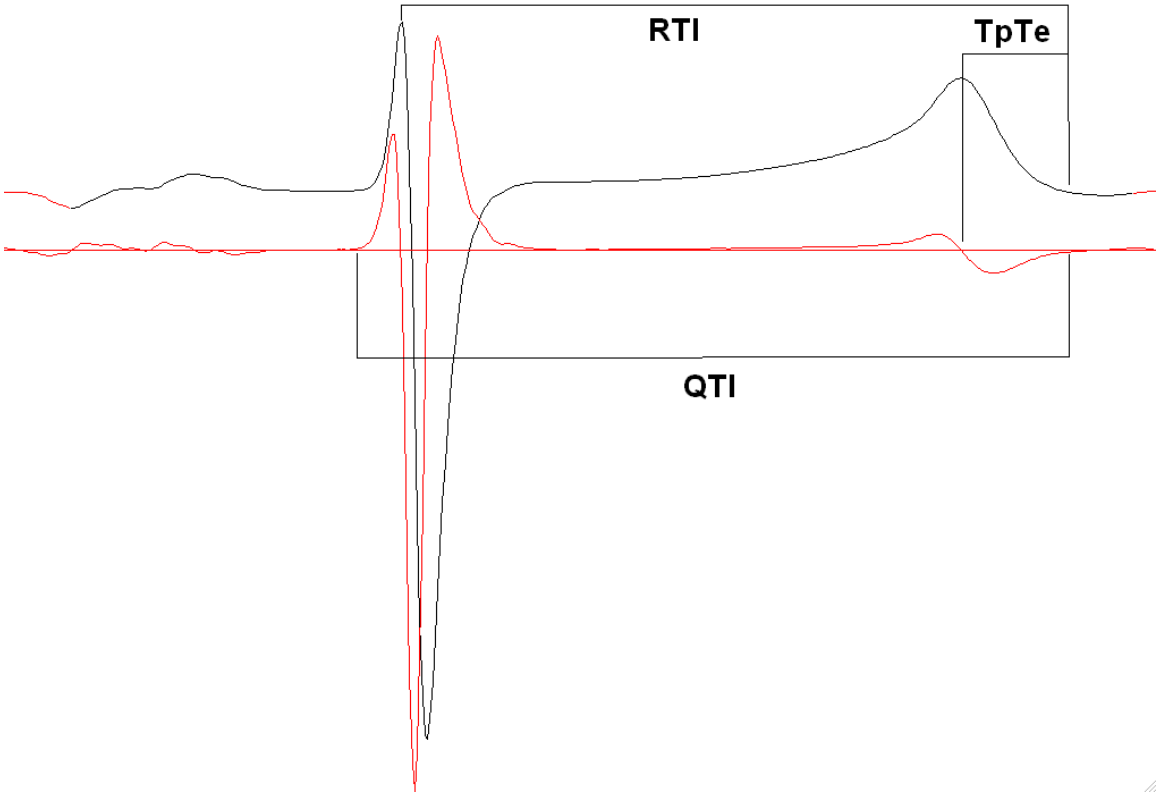
Figure 2.0 (cont'd)



DATA ACQUISITION & ASSESSMENT

Blood pressure and electrophysiologic data were acquired and digitally stored using EMKA-Iox (v1.8.9.9). Hemodynamic measurements used in this dissertation included *d*P/*dt* and peak LVP (LVP_{peak}). These left ventricular pressure waveform analysis was performed on EMKA-ECGAuto version 2.5.1.31 (EMKA Technologies, Paris, France). For electrocardiographic analysis, ECGAuto utilizes a shape-based algorithm to identify the beginning and ending of fiduciary points and related time periods. Analysis was considered successful when ≥ 80% of beats had successful fiducial point recognition for ECG and blood pressure waveforms. The RT interval (RTI) was substituted for the QT interval to improve on the inherent imprecision of marking the beginning of the Q-wave fiduciary point. The R-wave (i.e. first point in RTI), peak of the T-wave (Tp) and the end of the T-wave (Te) were defined as the zero crossing of the first derivative function of the ECG (Figure 2.0). During analysis a high-pass filter set at 0.05Hz and a low-pass filter set at 100Hz was applied.

Figure 2.1 Image of a single representative beat showing the ECG (black) with the corresponding first derivative, *d*V/*d*t (red). The time intervals corresponding to the QT interval (QTI), RT interval (RTI), and T-peak to T-end (TpTe) are shown.



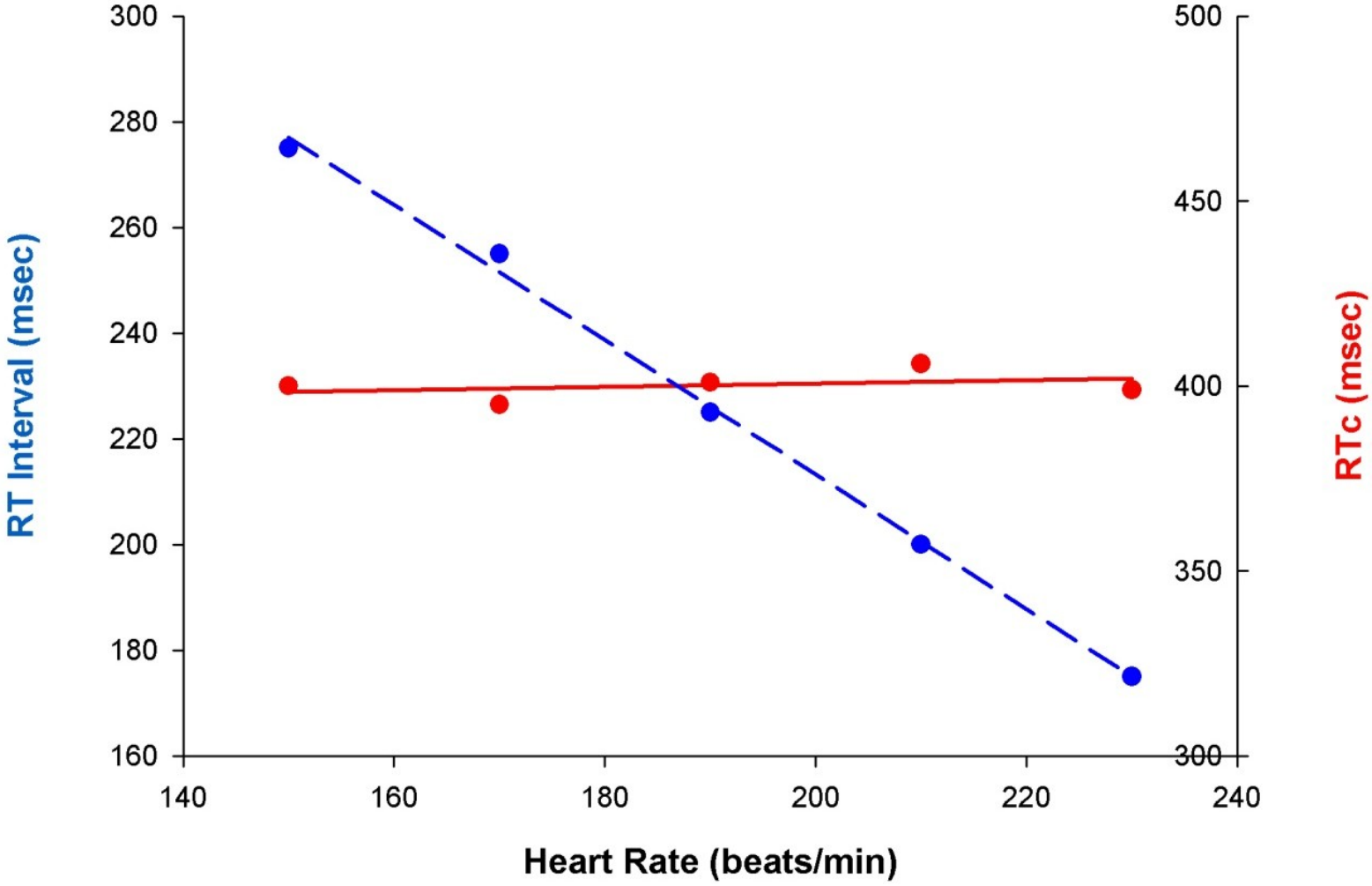
QT INTERVAL ASSESSMENT

Clinically, repolarization is measured using the QT interval of the electrocardiogram. Replacing the QT interval with the RT interval (peak of R-wave to T-end) has the advantage of being able to easily mark the beginning of the interval and avoids a potential measurement error in QT interval determination. However, as illustrated in Figure 2.1, the RT interval leaves out a portion of the QT interval corresponding to a section of the QRS duration. The QRS duration is the portion of the ECG that is a surrogate for whole heart depolarization. In any given complex, the QT and RT interval should be similar, and if they are not, it suggests a measurement error from alterations in depolarization rather than repolarization. For simplicity, the term QT is used in the remainder of this dissertation to refer to either QT (other studies) or RT (these studies) intervals.

QT INTERVAL CORRECTION

The duration of repolarization is heart rate dependent; the faster the ventricular rate, the shorter the duration of repolarization. The QT interval has been mathematically related to the proceeding cardiac cycle length in an effort to correct for changes in heart rate. These correction formulas were developed so that QT intervals, measured at various heart rates in humans or animal models, could be conveniently compared (Figure 2.2). The heart rate corrected QT interval has been validated utilizing experimental (Vincze et al., 2008; Farkas et al., 2009) and clinical (Schoenwald & Isaacs, 1974) data.

Figure 2.2 Hypothetical data for absolute QT interval (blue) acquired over a heart rate range of 150 to 230 beats/min, and the corresponding idealized corrected QT (QTc) that remove the effect of heart rate (red).



A corrected QT interval will complement absolute QT interval reporting. Since an effective parametric correction formula has not been validated for pigs, a linear correction scheme based on the observed slope of the relationship between heart rate and QT intervals as described in the following equation was used:

$$QTc = QT + ((1 - RR) * |slope|)$$

This approach works well for an individual pig; however, the slope term was highly variable among pigs. As a result, a single linear correction formula could not be used for these studies. Therefore, in order to understand the heart rate / QT interval relationship in domestic pigs more fully, the QT interval was assessed in isoflurane anesthetized pigs, compared to non-anesthetized caged pigs. Commonly applied population-based formulas (29 in total) were applied to ECG data derived for a period of 14 hours, while the animal was isolated and caged. The formulas were applied to the ECG for the same animal under isoflurane-based anesthesia. The heart rate / QT interval response observed in these caged animals was compared to the QT interval response observed in anesthetized pigs undergoing sequential pacing in a step-wise pattern from 150 - 230 beats/minute. The QT analysis performed on caged pigs was heart rate limited; to 150 – 230beats/minute. The heart rate / QT interval of caged pigs was also evaluated using the QT interval of the entire observed heart rate range. The 29 correction formulas referred to above were applied to each data set. Results from this study are summarized in Appendix A of this dissertation.

The efficacies of the corrections were assessed as the slope of the residual QTc versus heart rate relationship. Perfect correction is indicted by a residual slope of zero. The magnitude of the error was strongly influenced by the success in attaining a residual slope of zero. As the heart rate deviates from 60 beats/min, the greater the magnitude of the error. Slopes at heart rates of 150 beats/min or greater would correlate to an unacceptable risk if they resulted in errors greater than 6 msec (King et al. 2006).

The initial evaluation using these formulas yielded results suggesting that there is a high degree of intra and inter-animal variability in correction efficacy. The error introduced by using the correction formulas was unacceptable for experimental studies due to this large variation.

Therefore, the absolute QT intervals were used when comparing repolarization parameters among pacing types. For the evaluation of dofetilide pharmacokinetics (Chapter 4), and the evaluation of sinusoidal and random tachypacing with dofetilide treatment (Chapter 5), fixed-rate pacing was used before and after periods when sinusoidal or random tachypacing did not occur. This use of fixed-rate pacing stabilizes the heart rate and alleviates the need for the use of heart rate correction formulas.

ASSESSMENT OF REPOLARIZATION DISPERSION

The interval from the peak of the T-wave to the end of the T-wave (T_pT_e) can serve as an index of transmural dispersion of repolarization (Antzelevitch, 2001). Increased

transmural dispersion of repolarization has been proposed as a marker for proarrhythmic risk in experimental animals (Yan & Antzelevitch, 1998; Antzelevitch & Oliva, 2006; Antzelevitch, 2007; Wu et al., 2008) and in clinically-oriented studies (Shimzu et al., 1997; Yamaguchi et al., 2003; Zhang et al., 2008; Letsas et al., 2010).

SECONDARY MEASURES OF REPOLARIZATION

QT ADAPTATION

Following the termination of fixed, sinusoidal, and random pacing, the QT interval parameter was examined and compared as a function of time in each of the pacing protocols. The following curve-fit equation was applied to fit the QT interval adaptation:

$$y = a + bx^2 + cx^{0.5}$$

this polynomial-type equation has inherent challenges for interpretations due to the generation of two dependent or collinear parameters (i.e. b and c). The “c” coefficient primarily affects early gain. The “b” coefficient primarily affects late gain and acts as a *bending* parameter to move the otherwise upward movement of the early phase of the curve to become more horizontal. Factor “c” has fifty-times more weight in altering the shape of the curve than the “b” coefficient. Factor “c” predominates throughout adaptation; however the “b” coefficient acts as a bending element, a situation that was

nonexistent for the first 30 beats. Therefore, “b” has negligible effects until the curve approaches the near-horizontal, new steady state.

In these studies the focus was on comparing the early QT adaptations across different treatment pacing types. Therefore, because the “b” coefficient does not have a strong effect during the early portion of the curve, the “c” coefficient was the primary outcome of interest.

QT (RT) INTERVAL MEMORY ASSESSMENT

The rate of change in the QT interval was evaluated as a function of the preceding ventricular cycle length. This short term “memory” was assessed using Poincare plot analysis of the QT as a function of consecutive R to R intervals (i.e. QT for RR_n versus RR_{n-1}). The following formula, where BVR stands for the beat-to-beat variability of repolarization, was applied to measure “memory”:

$$BVR = \frac{QT_{n-1} - QT_n}{\sqrt{2}}$$

The repolarization variance was examined based on a QT variability index ($QTvi$) and has been validated as a heat rate dependent tool for the assessment of proarryhmic risk (Soyka et al., 1990; Berger et al., 1997; Bilchick et al., 2004; Nolan et al., 2004). The mean heart rate ($\overline{HR}$) and mean QT interval ($\overline{QT}$) with the corresponding variance

(HRv, QTv) during an epoch of interest will be used according to the following formula:

$$RTvi = log_{10}\left(\frac{QTv/\overline{QT}^2}{HRv/\overline{HR}^2}\right)$$

DOFETILIDE ASSESSMENT

To assist interpretation of the effect of dofetilide on cardiac repolarization dynamics, dofetilide serum concentrations were estimated using an extraction technique, adapted from Walker et al. (Walker et al., 1991 and 1996) followed by High Pressure Liquid Chromatography (HPLC). Dofetilide (Milwaukee, WI) obtained from Sigma-Aldrich was dissolved in sterile water with 0.5N sodium hydroxide for subject administration and HPLC standard curve determination. Sequential blood withdrawals (8ml) were performed, through the femoral vein, correcting for the intravascular volume deficit with normal saline (0.9%). The whole blood was allowed to coagulate for 30 minutes and then centrifuged. The serum was stored at -80^{O} C for further analysis. Serum was used for chromatographic analysis due to its observed higher recovery compared to heparin or EDTA based plasma collection. Samples (500ul) were alkalized with 0.2M sodium borate buffer (1ml – pH 9.0) into tert-butyl methyl ether (2.5ml). The solution was centrifuged and evaporated with nitrogen and suspended in 100ul acetonitrile/20mM ammonium phosphate buffer (33:67). A 20ul aliquot was injected into the HPLC analytical Phenomex Luna 5u C-18 (250x4.6mm) column. Quantified data

was interpreted at 230nm. The extraction efficiency and limit of detection was established based upon an experiment using serum, exposed to excess dofetilide, just prior to the execution of the extraction procedure (Chapter 4). The chromatograms were integrated with Waters Millennium system (2000 version).

DATA & STATISTICAL ANALYSIS

Throughout this dissertation, results are presented in customary units unless otherwise specified. Units of heart rate are beats per minute (beats/min), pressure in millimeters of mercury (mmHg), myocardial contractility as a first derivative of pressure with respect to time (dP/dt), and time in minutes (min) or milliseconds (msec). Data are presented using the mean as the measure of central tendency and standard error of the mean (SEM) as the measure of dispersion.

Some comparisons involve repeated measures over time. A repeated measures analysis of variance (ANOVA) was used for these analyses. In some cases these repeats over time span substantially different physiologic conditions such as unpaced, pacing at 150 beats/min, and tachypacing. In these instances, repeated measures ANOVA analyses were restricted to data within a condition. For example, data were occasionally collected during baseline, during pacing at 150 beats/min, during test tachypacing, and during the post-tachypacing period. Rather than consider all of this a one-factor repeated measures design spanning all experimental conditions, analyses were restricted to like conditions (i.e. repeated measures during test tachypacing).

When comparisons involve time or groups as factors, differences were assessed by a repeated measures ANOVA with Fisher's Least Significant Differences used for post hoc testing if needed. Interactive effects were tested using linear contrasts. When only two groups or times were compared, a student's t-test was considered sufficient for the analysis. A statistically significant difference was defined as a nominal type 1 error rate of less than 5% ($p<0.05$).

CHAPTER THREE – THE EFFECT OF TACHYCARDIC IRREGULARITY ON VENTRICULAR REPOLARIZATION DYNAMICS IN *NORMAL* DOMESTIC PIG

INTRODUCTION

It is difficult to create a controlled study in humans with atrial fibrillation due to a milieu of disparate intra-subject characteristics. This is complicated by ethical limitations on human experimentation. Therefore, controlled studies in animals were needed to more fully manipulate cardiac rate and regularity in order to evaluate and describe its effects on cardiac repolarization. Domestic pigs were selected for study in part because they are suitably sized to allow for catheter manipulation and invasive electrophysiology studies. More importantly, pigs are similar to humans with regard to major depolarization and repolarization currents. Current animal models that mimic atrial fibrillation have several undesirable characteristics. They rely on the creation of a structurally abnormal heart with drugs, vascular occlusion or a combination of both. These hearts are structurally abnormal and often require the co-treatment with drugs that frequently alter the hearts electrophysiology. These features, coupled with the unpredictable and often transient nature of the atrial fibrillation necessitates a more controlled animal model. Our model of temporary atrial overdrive pacing was designed to study the effects of tachypacing on the hearts' repolarization. It has the advantage of being able to control the onset and termination of tachypacing independent of additional

drugs. One limitation is that it does not accurately mimic the atrial hemodynamics of naturally occurring atrial fibrillation: It has a partially preserved atrial contraction.

Reductionism approaches a scientific question relying on the assumption that the behavior of a component tissue or cell, when extrapolated to the whole organism, will organize in a non-complex way. Reductionisms' approach has added considerably to the body of scientific knowledge and has posed many interesting hypotheses. These hypotheses can only be properly explored and found to be valid for an organism if they are tested in an organismal model. Indeed, mathematical models are unavailable to explore this complex system because modeling requires the assumption that the parts of a system summate to the whole: An assumption that is not currently consistent with reality. Whole animals also have an intact central nervous system and mechanoelectric feedback operating through both intra and extra-cardiac ganglia. Whole animal studies, by using minimally invasive techniques, can control extra-cardiac effects that play a role in modifying cardiac repolarization.

This arm of study is designed to study the effects of fixed rate, sinusoidal and random pacing on typically used repolarization parameters and how these parameters change over time. This line of study is designed to test the hypothesis that transient irregular, as compared to fixed and sine wave overdrive atrial tachypacing alters ventricular repolarization dynamics.

SPECIFIC METHODS

Domestic pigs (n=7) were anesthetized with isoflurane and instrumented as previously described. The basic protocol is outlined in Figure 3.0. Animals were allowed to develop a stable hemodynamic (blood pressure and heart rate) baseline after animal preparations were completed. Thereafter, baseline data were collected for 30 minutes. High right atrial pacing was performed in order to simulate the random ventricular response observed during atrial fibrillation, the fixed response as seen with fixed-rate tachycardias and sinusoidal to control for the variation of random pacing. This technique of tachypacing was used to test the main hypotheses in this chapter: random pacing alters ventricular repolarization differently than fixed or sinusoidal pacing. The advantage of high right atrial pacing is that the depolarization current travels though the His-Purkinje system similarly to atrial fibrillation. However, this is a limited model of atrial fibrillation because it does not account for the observed loss of atrial contraction during atrial fibrillation. Pacing stimuli were applied to high right atrium for 60 minutes and randomized into three groups: Fixed, sine and random pacing. Each subject received all three pacing treatments. Fixed rate pacing was delivered at a rate of 200 beats/min (CL: 300 milliseconds) while sinusoidal pacing was delivered at a mean rate of 200 beats per minute with a frequency of 0.25 Hertz and a range of 60 beats/min. Random pacing was a uniform pseudo-random sequence distributed around a mean of 200 beats per minute with a variance similar to that of a sine wave pacing protocol (Figure 3.1).

Figure 3.0 Protocol to assess repolarization alteration following fixed, sinusoidal or random pacing, applied to each animal in random sequence.

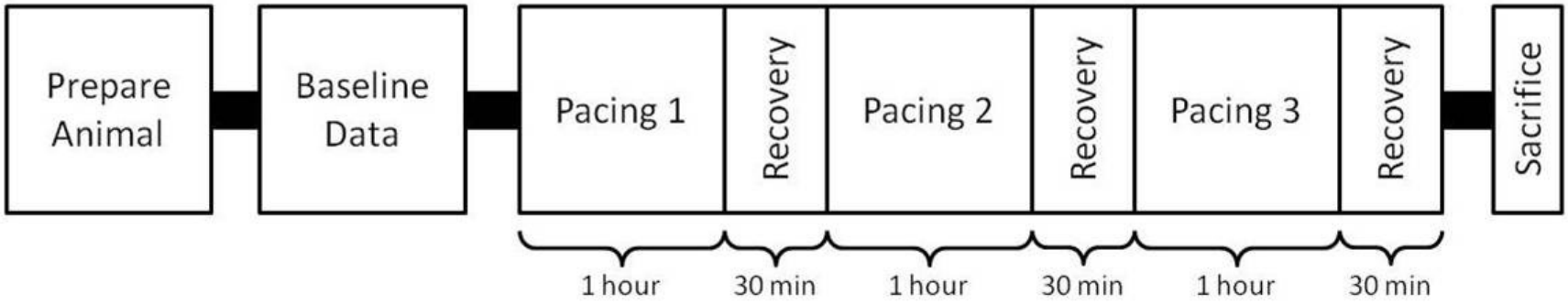
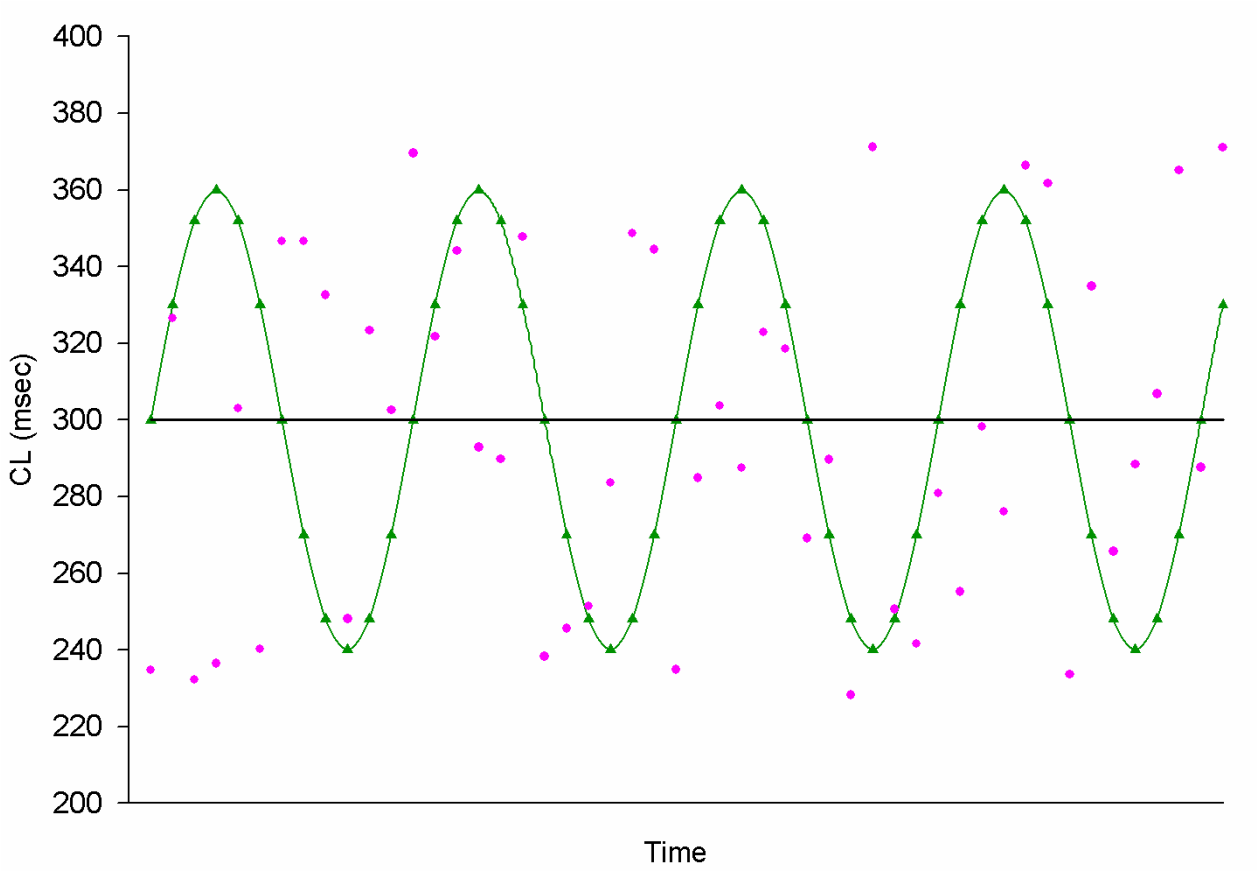


Figure 3.1 Diagrammatic representation of high right atrial pacing paradigms ($\overline{CL}$:300) showing fixed (black), sinusoidal (green) and random (pink) tachypacing.



DATA

CHARACTERIZATION OF AN EXPERIMENTAL MODEL FOR STUDYING CARDIAC REPOLARIZATION

Electrical and mechanical coupling was confirmed during pacing (Figure 3.2). Ventricular rates were selected based on being reasonable physiologic rates encountered during atrial fibrillation in the domestic pig as well as being higher than the resting heart rate of anesthetized pig; however, not excessively high as to induce atrioventricular nodal blockade.

EVALUATION OF THE EFFECT OF REPETITIVE PACING ON THE PRE-PACING BASELINE INHERENT VENTRICULAR RATE

Having shown the feasibility of high right atrial pacing to induce fixed, sine wave and random ventricular capture, we next apply this to a cohort of animals. The QT interval changes with the heart rate in a reciprocal fashion. Therefore tachypacing for a period will alter the QT interval. The order of protocols (fixed, sine wave or random) within each experiment (pig) was randomized. Due to the potential of the heart rate to fluctuate based on the preceding tachypacing protocol, it is important to check whether inherent ventricular rate changes prior to each pacing protocol. The summary of this analysis is contained in Figure 3.3 and Table 3.0. There was no significant difference in pre-pacing cycle lengths between each pacing type ($p=0.39$): Pre-fixed pacing (PreFP), Pre-sine wave pacing (PreSP) and Pre-random pacing (PreRP).

Figure 3.2 Raw data of transition from fixed high right atrial pacing to unpaced depicting an electrocardiogram (ECG), electrograms (EGM), right ventricular pressure (RVP), peripheral blood pressure (BP), left ventricular pressure (LVP), and a monitoring channel for pacing (Stim).

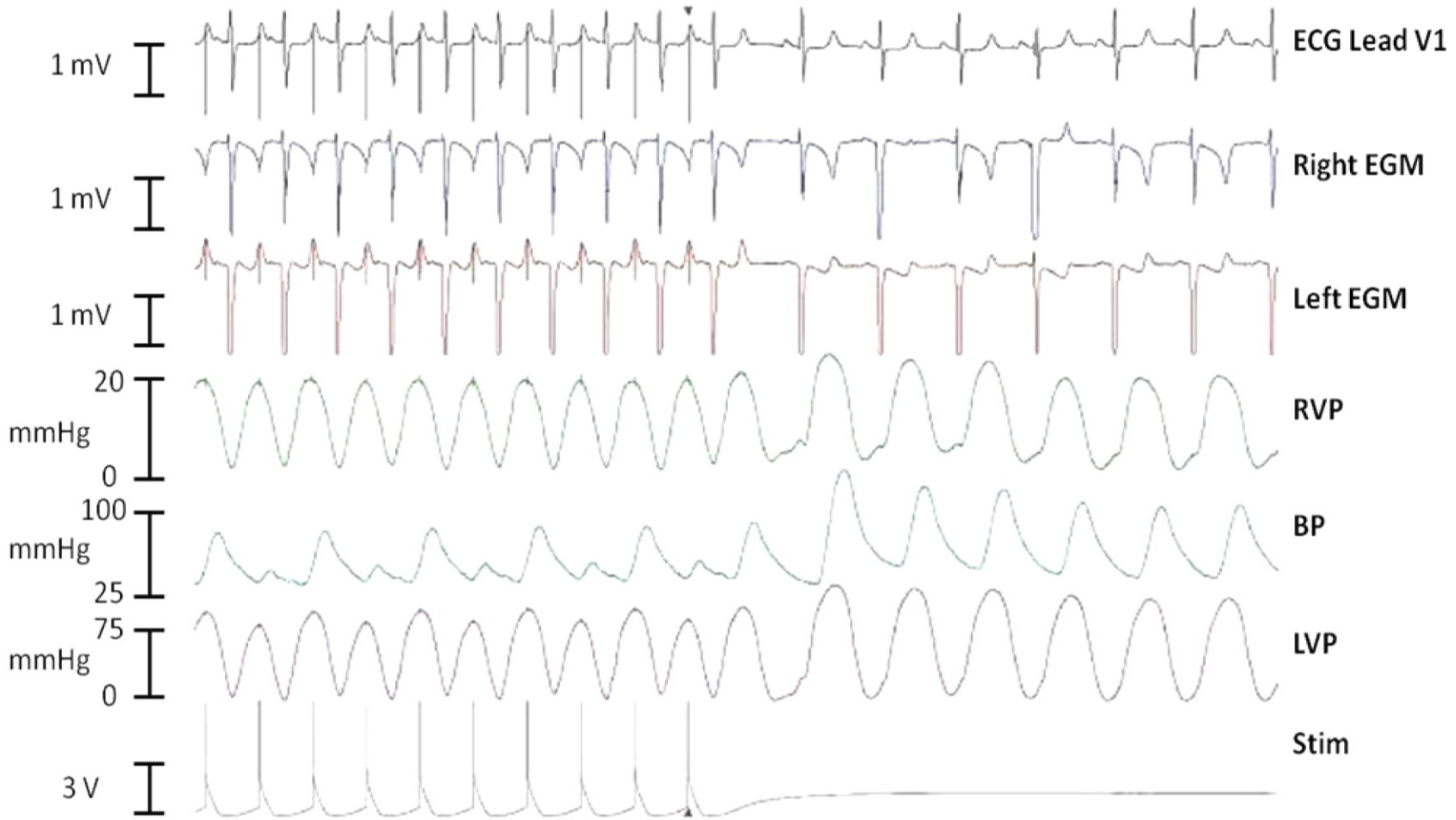


Figure 3.3 Pre-pacing cycle lengths comparing Pre-fixed (PreFP), Pre-Sinusoidal (PreSP), and Pre-random paced (PreRP) between groups (n=7). Vertical bars represent the SEM. (p=0.39)

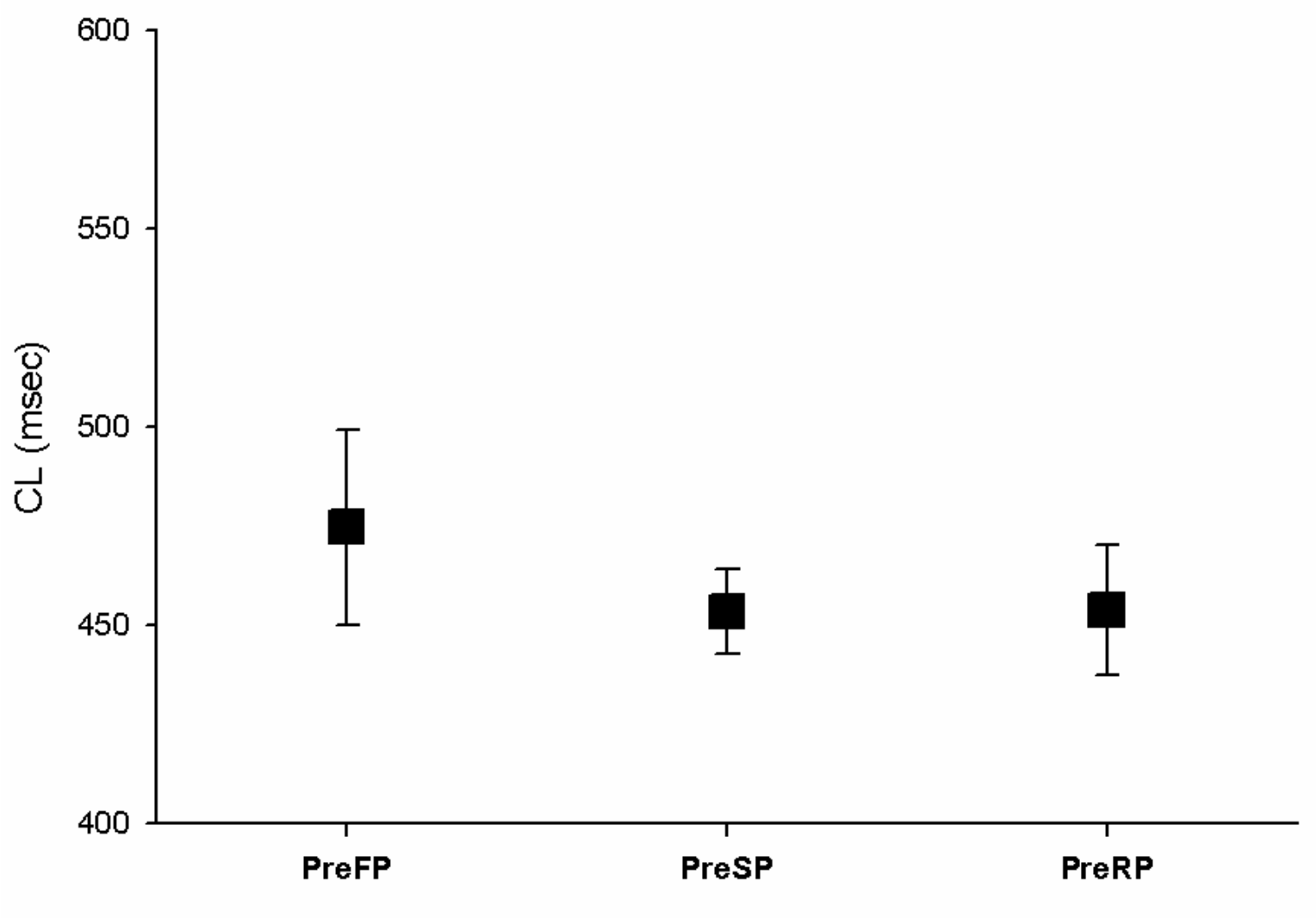


Table 3.0 Statistics table of results from comparing cycle lengths (msec) between each type of pacing (n=7). (p=0.39)

Pacing Type	Mean	Standard Error	-95% CI	+95% CI
Pre-Fixed Pacing	474.7	24.5	416.8	532.5
Pre-Sine Wave Pacing	453.4	10.7	428.2	478.7
Pre-Random Pacing	453.9	16.3	415.3	492.5

Heart rate changes with the QT interval in a reciprocal fashion. Therefore tachypacing for a period will alter the QT interval. Therefore, it is important to determine if and to what extent the QT interval is itself affected by tachypacing. Specifically we are interested in the potential alteration of the intrinsic ventricular cycle length before and after pacing so that for future studies we can more fully evaluate the QT interval while providing consideration for the magnitude QT interval changes brought about from heart rate changes. Here I hypothesize that ventricular heart rate remains unchanged before and after fixed, sinusoidal and random tachypacing. The summary of this analysis is contained in Figure 3.4 and 3.5. The fixed ($p=0.18$), sine ($p=0.21$), and random ($p=0.16$) pre-pacing cycle length was not significantly different than post-pacing. Each post-pacing 30-second intervals did not show any significant difference within the group or between groups ($p=0.41$).

Figure 3.4 Average heart rate (beats/min) before and after fixed, sinusoidal (Sine), and random tachypacing (n=7). Data was averaged over 5 minutes calculated just before and after tachypacing.

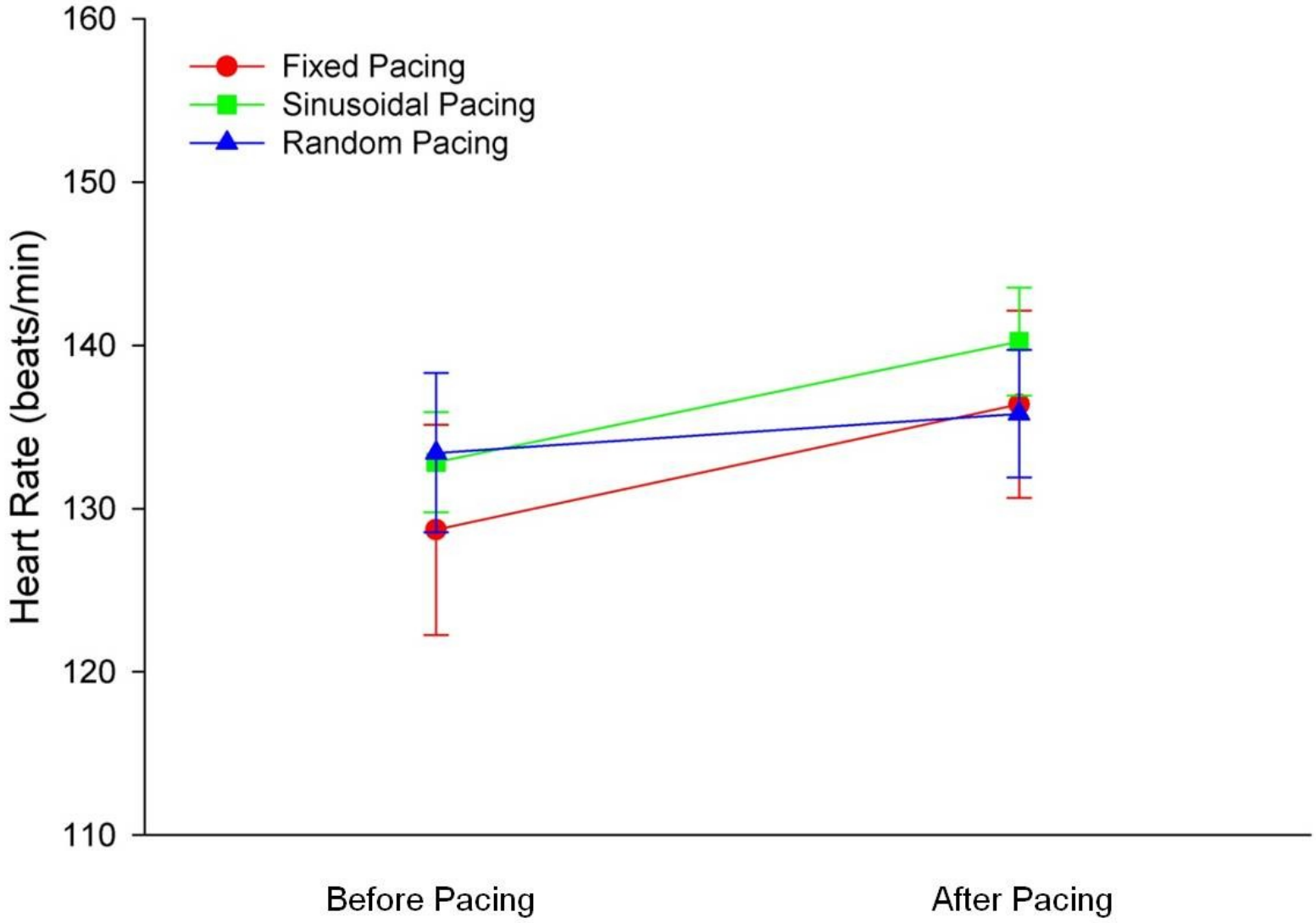
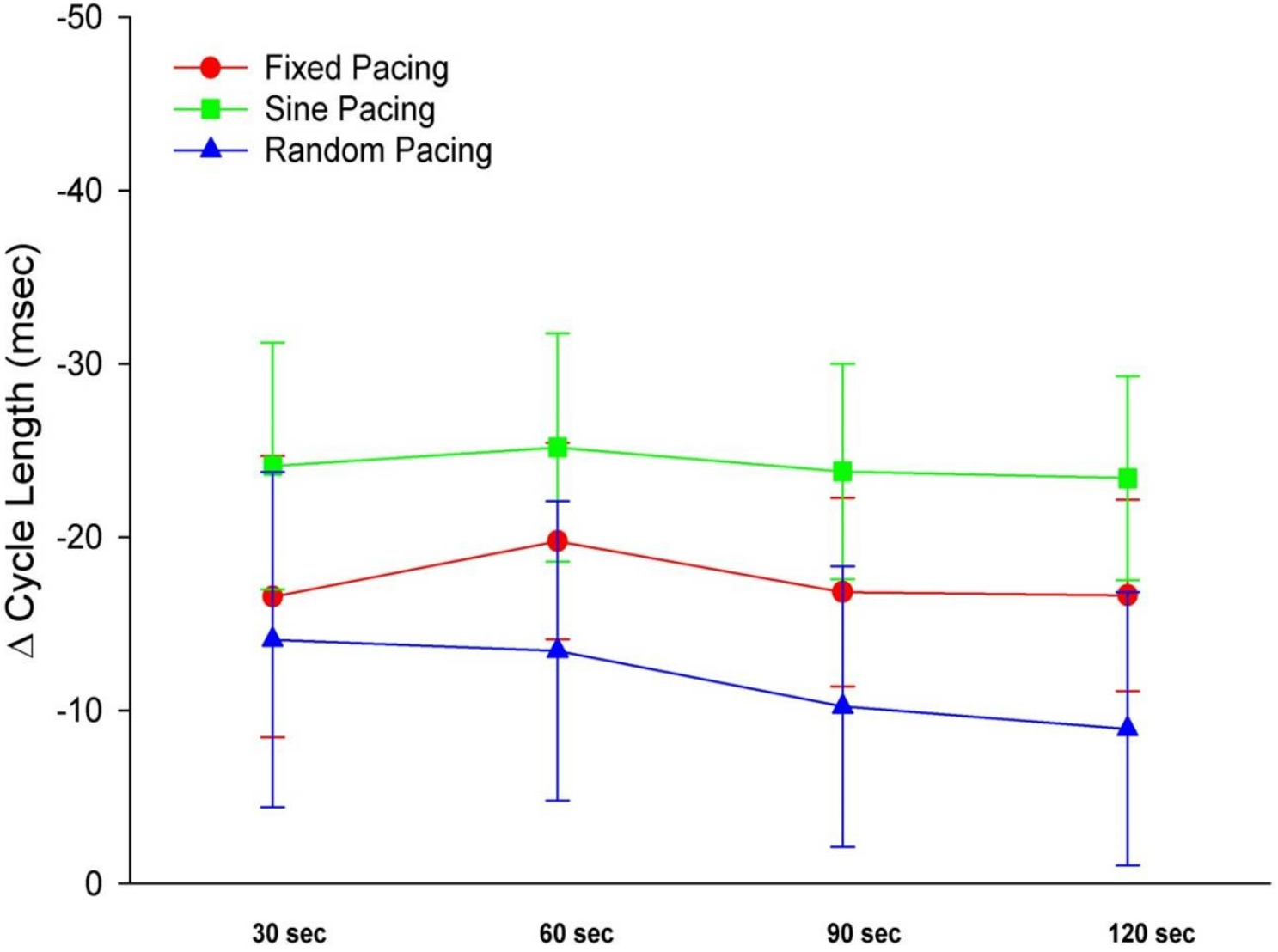


Figure 3.5 Change in fixed, sinusoidal (sine) and random pacing cycle lengths (CL) compared to the pre-pacing interval at 30-second post-pacing intervals (n=7). (p=0.41)



EVALUATION OF THE EFFECT OF TACHYPACING ON THE QT INTERVAL AND T_pT_e SEGMENT

Using statistical methods previously described, fixed, sinusoidal, and random tachypacing does not differentially alter the QT interval during or shortly after the termination of tachypacing. The T_pT_e segment duration was evaluated as a surrogate for transmural dispersion of repolarization. During and after periods of tachypacing the T_pT_e segment duration was not differentially significantly altered (Table 3.1).

EVALUATION OF QT DYNAMIC DURING AND AFTER TACHYPACING

Poincare plots provide a visual representation of beat-to-beat variability of repolarization (BVR). Figure 3.6 is a summary of the data in 7 animals for the QT interval as a function of the previous QT interval. The average BVR for fixed (0.40 ± 0.056), sinusoidal (0.57 ± 0.203), and random (0.40 ± 0.061) was not significantly different between fixed and random ($p=0.95$) or sinusoidal and random ($p=0.37$). Figure 3.7 provides a summary for the QT adaption following the termination of tachypacing over the first 250 beats. The curve fit was applied as previously outlined and the summary of the statistics is contained within Table 3.2. Figure 3.8 provides a graphical reorientation of the "c" coefficient, the parameter of prime importance due to its influence on the early gain of the QT response. This and the other coefficients were not significantly different. The QT variability index (QTvi) is summarized it Table 3.3. Evaluations were for 5 minutes of data just prior and after tachypacing, at 15, 30 and 60 minutes during

tachypacing of one hour. These pre-pacing (p=0.33), post-pacing (p=0.19) QTvi and the group QTvi during pacing (p=0.14) was not significantly different (Figure 3.9).

Table 3.1 RT interval (RTI), QT interval (QTI), and T_pT_e (TpTe) values (n=7) computed over 5 minutes at selected periods just before (Pre) and after (Post) tachypacing and the end of one hour of tachypacing (Post).

		RTI	SEM	QTI	SEM	TpTe	SEM
Fixed	Pre Tachypacing	252.7	13.9	272.7	13.6	43.9	2.9
	During Tachypacing	202.9	11.4	219.4	10.3	48.4	10.4
	Post Tachypacing	232.0	10.4	251.2	10.4	43.6	3.1
Sinusoidal	Pre Tachypacing	239.8	9.7	259.5	9.7	42.9	3.1
	During Tachypacing	193.6	4.9	211.4	5.5	40.7	3.7
	Post Tachypacing	223.1	9.2	242.7	9.3	40.9	2.9
Random	Pre Tachypacing	247.4	6.9	266.9	7.6	41.9	3.4
	During Tachypacing	202.5	6.0	220.8	6.3	41.4	5.1
	Post Tachypacing	231.8	8.0	250.8	9.0	39.9	3.5

Figure 3.6 – Poincare plots as a visual representation of the QT interval beat-to-beat variability of repolarization (BVR) each representing the RT interval as a function of the previous RT interval in a single subject (n=7). [The text in this figure is not meant to be decipherable, but is for visual reference only.] *Refer to Appendix B for individual decipherable graphs.*

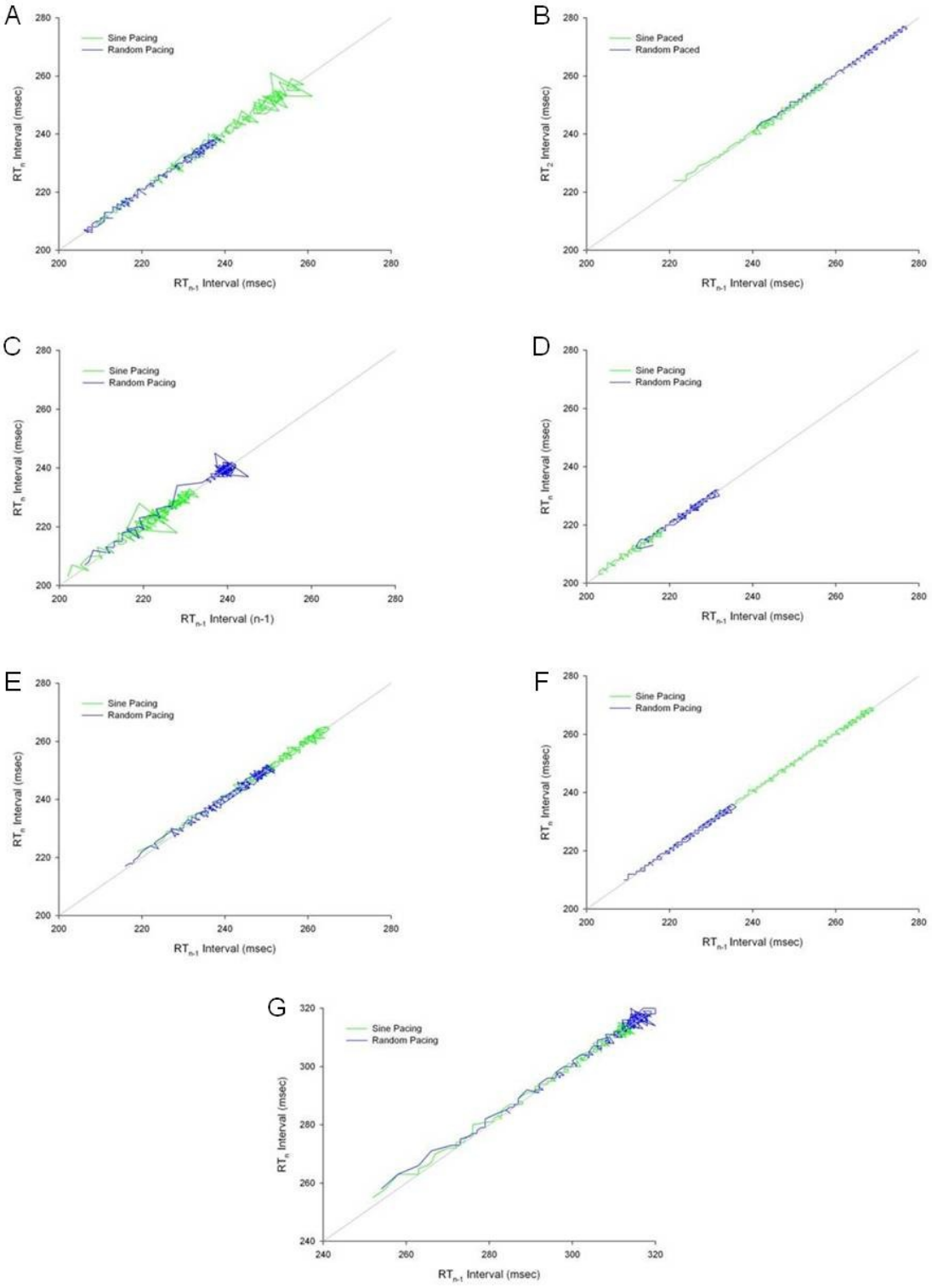


Figure 3.7 QT (RT) interval adaptations over 250 beats following the termination of fixed, sinusoidal (Sine), and random tachypacing. Each graph represents a single animal (n=7). [The text in this figure is not meant to be decipherable, but is for visual reference only.] *Refer to Appendix C for individual decipherable graphs.*

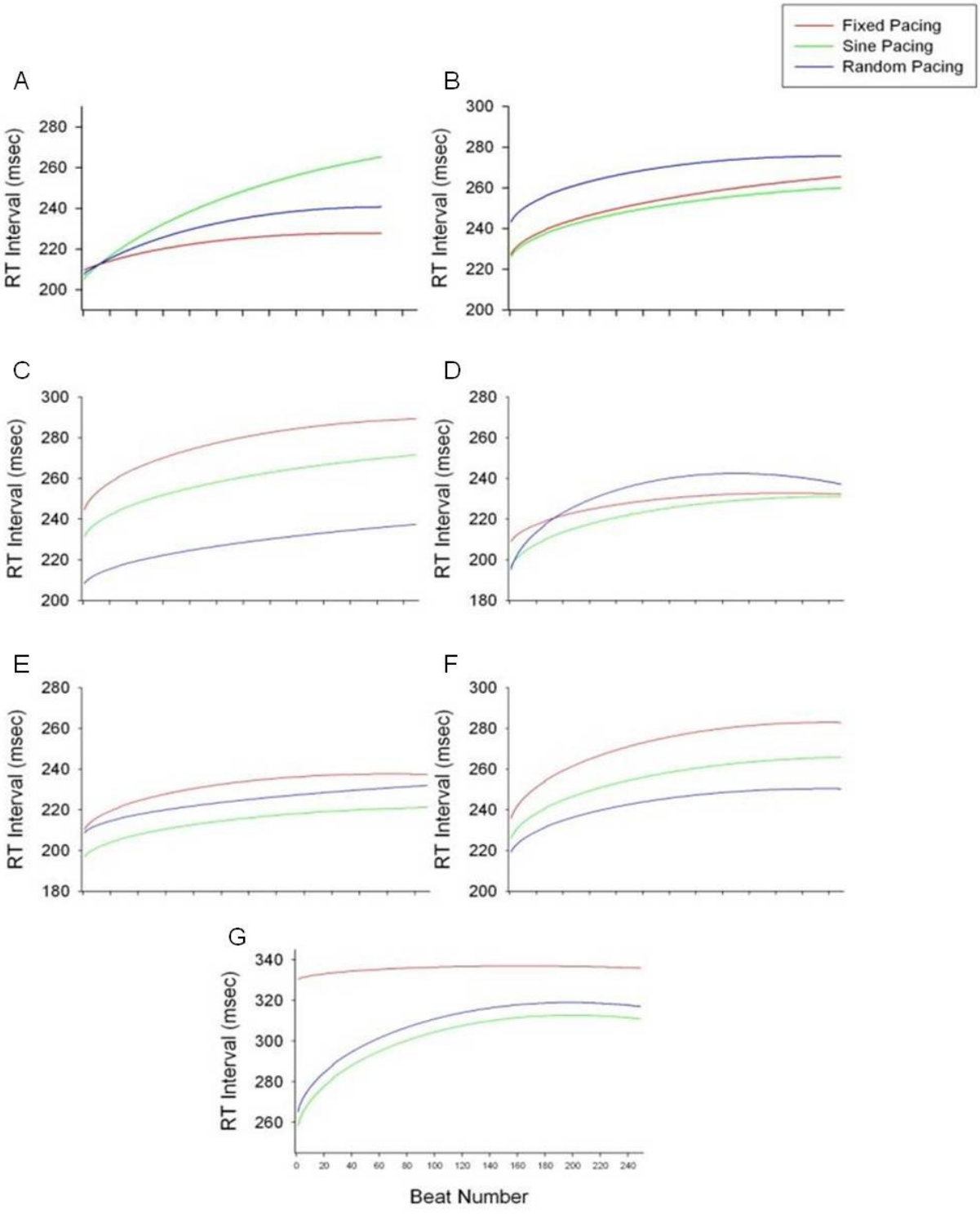


Table 3.2 The QT (RT) interval curve fit data table for factor A (A), B (B), C (C) and linear coefficient of determination, R^2 (RSQR) in 7 animals. P-Value[1] refers to paired t-test for random versus fixed. P-Value[2] refers to paired t-test for random versus sinusoidal.

	RANDOM	SEM	FIXED	SEM	P-Value [1]	SINUSOIDAL	SEM	P-Value [2]
A	214.68	10.25	233.66	17.07	0.11	212.38	10.32	0.67
B	-0.00023	0.00008	-0.00018	0.00003	0.41	-0.00020	0.00005	0.32
C	3.64	0.61	2.83	0.44	0.43	3.84	0.64	0.69
RSQR	0.95	0.03	0.92	0.05	0.56	0.98	0.01	0.56

Figure 3.8 The QT interval curve fit “c Factor” comparison across the three (Fixed, Sinusoidal (Sine), and Random Pacing) treatment groups (n=7).

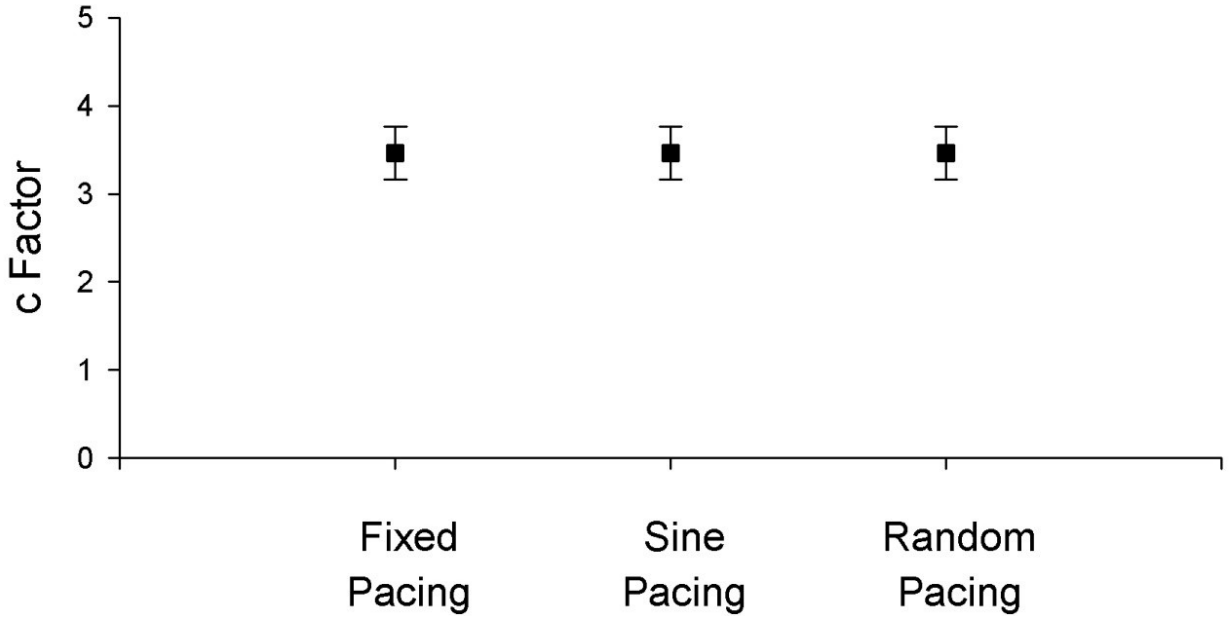
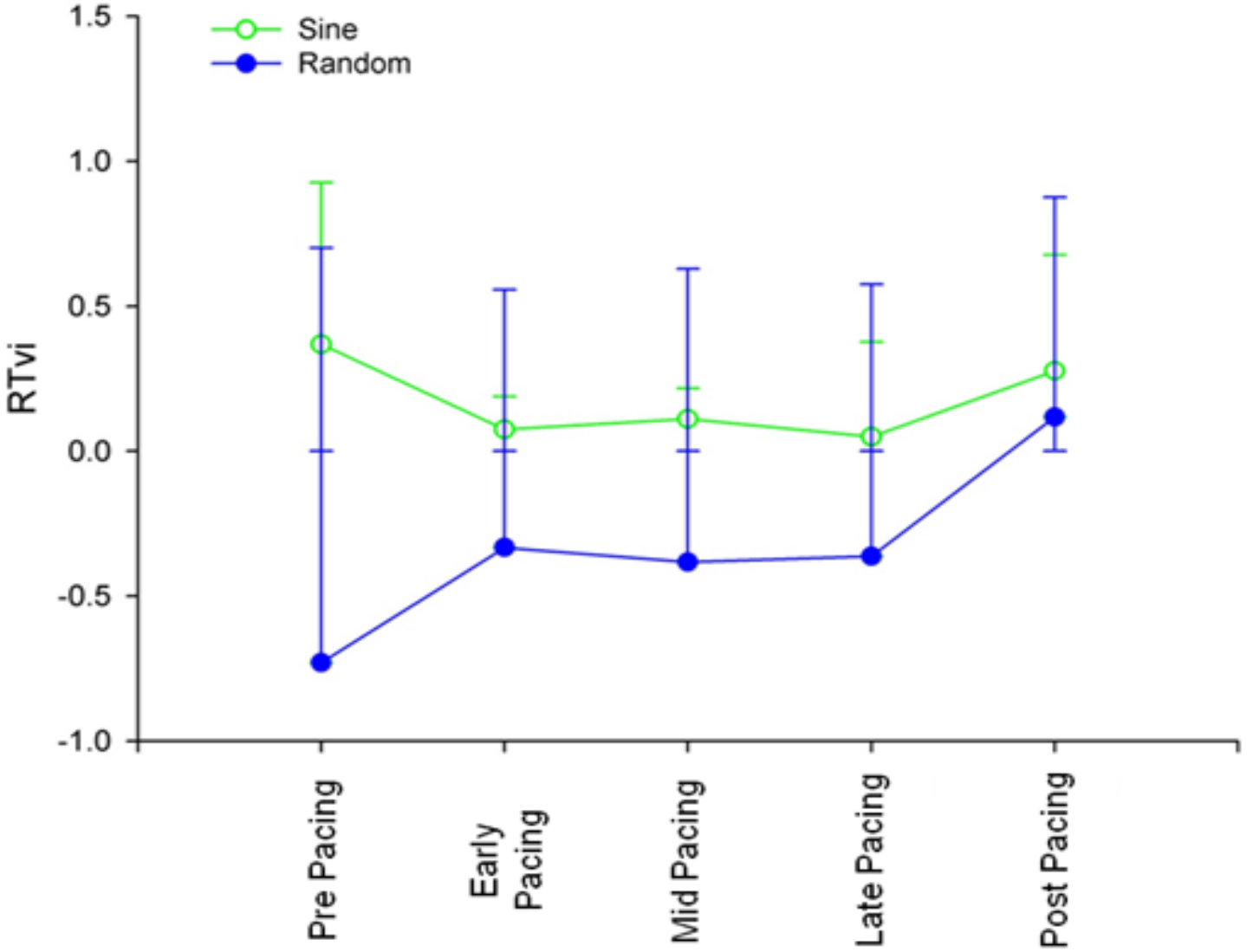


Table 3.3 QT interval variability computed over 5 minutes just before tachypacing began and ended, just after tachypacing, and during tachypacing at points centered at 15 and 30 minutes (n=7).

QTvi	Pre Tachypacing	SEM	Early Tachypacing	SEM	Mid Tachypacing	SEM	Late Tachypacing	SEM	Post Tachypacing	SEM
Sinusoidal	0.368	0.696	0.105	0.104	0.166	0.060	0.147	0.026	0.262	0.582
Random	-0.572	0.583	-0.496	0.050	-0.483	0.055	-0.473	0.048	0.072	0.598

Figure 3.9 QT variability index (RTvi) computed over 5 minutes at select center-points during the experiment corresponding to before, during (Early-15 min, Mid-30 min, Late-60 min), and after sinusoidal and random tachypacing (n=7).



OVERALL CONCLUSION

These experiments found that a comprehensive list of repolarization parameters is not differentially affected either during or shortly after fixed, sinusoidal, and random tachypacing.

CHAPTER FOUR – THE QT EFFECT AND PHAMRMACOKINETIC PROFILE OF DOFETILIDE IN TACHYPACED DOMESTIC PIGS

INTRODUCTION

Drug induced QT interval changes remain a concern that restricts potentially efficacious compounds from reaching the market (Malik, 2005). Heightened scrutiny from pharmaceutical regulators has slowed preclinical drug development; one-third of all drug withdrawals between 1900 and 2006 were a result of QT interval prolongation (Shah, 2006). This is due to the understanding that certain non-sedating antihistamine, antifungal, psychotropic and chemotherapeutic compounds are associated with arrythmogenic sudden death. These findings have spurred research trying to elucidate the mechanism of drug induced QT interval liability. Yet, the typical animal models aimed at understanding the mechanisms of QT interval-mediated sudden death have failed to adequately explain its underlying mechanisms (Dumotier & Georgieva, 2007). This suggests the need to expand studies to include other animal models.

In animal models, the QT interval is frequently used as a surrogate to measure the effects of compounds on cardiac ventricular repolarization. QT interval prolongation is associated with the development of repolarization-related arrhythmias such as Torsades (Pollard, 2010). Previous reports have suggested a varied pharmacokinetic response between humans, dogs and monkeys: species typically used in drug testing. Indeed,

humans given similar doses to monkeys are far less sensitive to the QT prolongation effects of dofetilide. Despite this, monkeys had a 3-fold lower exposure (i.e. AUC) than man. Dogs given 10 times the dose of either monkeys or humans had a similar QTc change to monkeys. The lower plasma exposure in monkeys seems to be related to an increased risk for repolarization-related arrhythmia (Haushalter et al. 2008) and is consistent to work in humans that shows a lower risk correlating with lower exposures (Allen et al, 2000). The fundamental pharmacokinetics underlying these observations may relate to altered bioavailability from oral administration or clearance effects (Smith et al., 1992).

Dofetilide is a VW Class III antiarrhythmic that is given clinically for the correction of life-threatening arrhythmias. It is known to cause QT interval prolongation and have a dose-dependent correlation with the risk for developing sudden cardiac death in humans and animal models. Altered repolarization by dofetilide is mediated through selective inhibition of the potassium current (I_K) via its inhibition of hERG ($I_{K,rapid}$) and KVLQT1/minK ($I_{K,slow}$) (Varro et al., 2004). Nearly all forms of drug liability risk associated with repolarization-related arrhythmia have been due to delayed rectifier inhibition (Kano et al., 2005; Witchel, 2011).

The findings from the previous studies described in this dissertation indicate that repolarization may not be altered as a result of short-term tachypacing in *normal*

domestic pigs. In the human clinical studies that suggested post-conversion vulnerability to RRAs, successful conversion of atrial fibrillation was often facilitated by the administration of I_{Kr} blockers. Therefore in order to test the hypothesis that I_{Kr} antagonism is involved in mediating these findings, dofetilide was chosen as the I_{Kr} blocker to use in these experimental subjects. There is a lack of information related to the pharmacokinetics of dofetilide in the domestic pig. As a result, a preliminary study was designed and undertaken to define these important features of the drug in this model. The objectives of this chapter are to determine the pharmacokinetic profile of dofetilide following a single intravenous injection and to examine the degree of repolarization prolongation introduced by the administration of dofetilide. We also are presenting data on factors that can play a modifying role on sudden cardiac death, including blood pressure, QRS duration, myocardial contractility and heart rate.

SPECIFIC METHODS

Domestic pigs (n=7) were anesthetized with isoflurane and instrumented as previously described. The basic protocol is outlined in Figure 4.0. Baseline data was collected for 15 minutes without pacing, and then continued for another 300 minutes during high right atrial, fixed rate pacing at 150 beats/min. The animals were then administered, through a peripheral ear vein, a 5-minute infusion of dofetilide (Sigma-Aldrich, St. Louis, MO) at a dose of 200mcg/kg. While pacing continued for 300 minutes, sequential 8ml blood

withdraws for serum continued according to the outline in Figure 4.1. After 5 hours, the pacing was terminated, and an additional 15 minutes of data was collected.

Figure 4.0 Protocol to assess the pharmacokinetic and pharmacodynamics response to a single bolus injection of dofetilide.

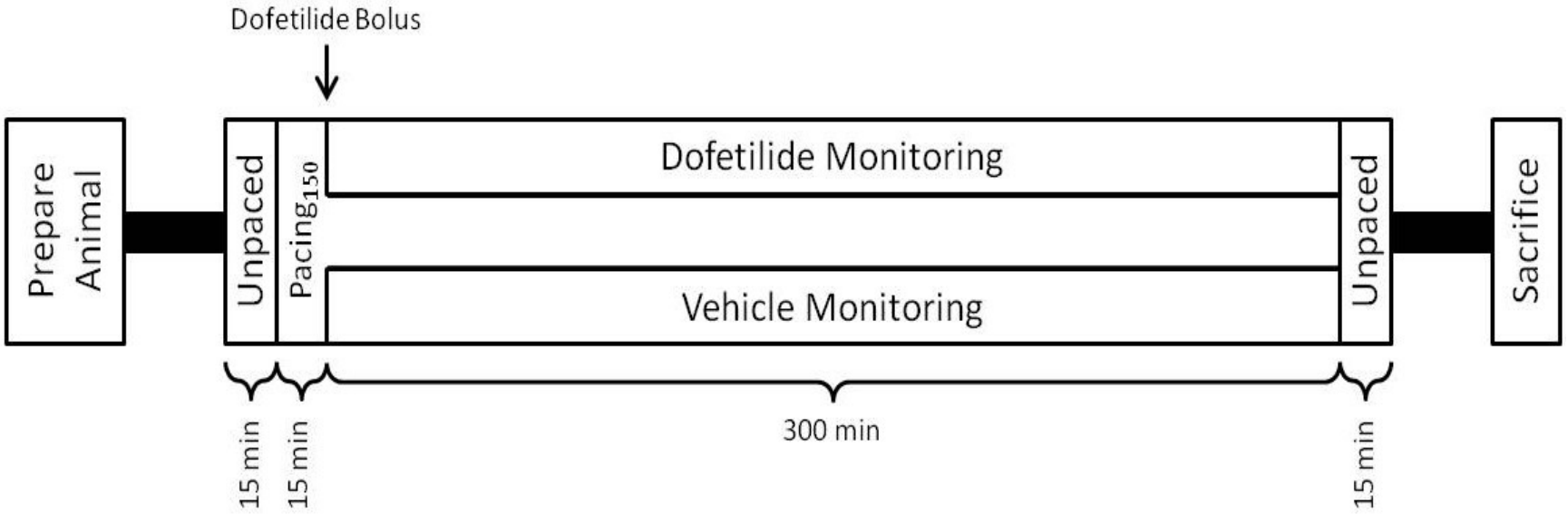
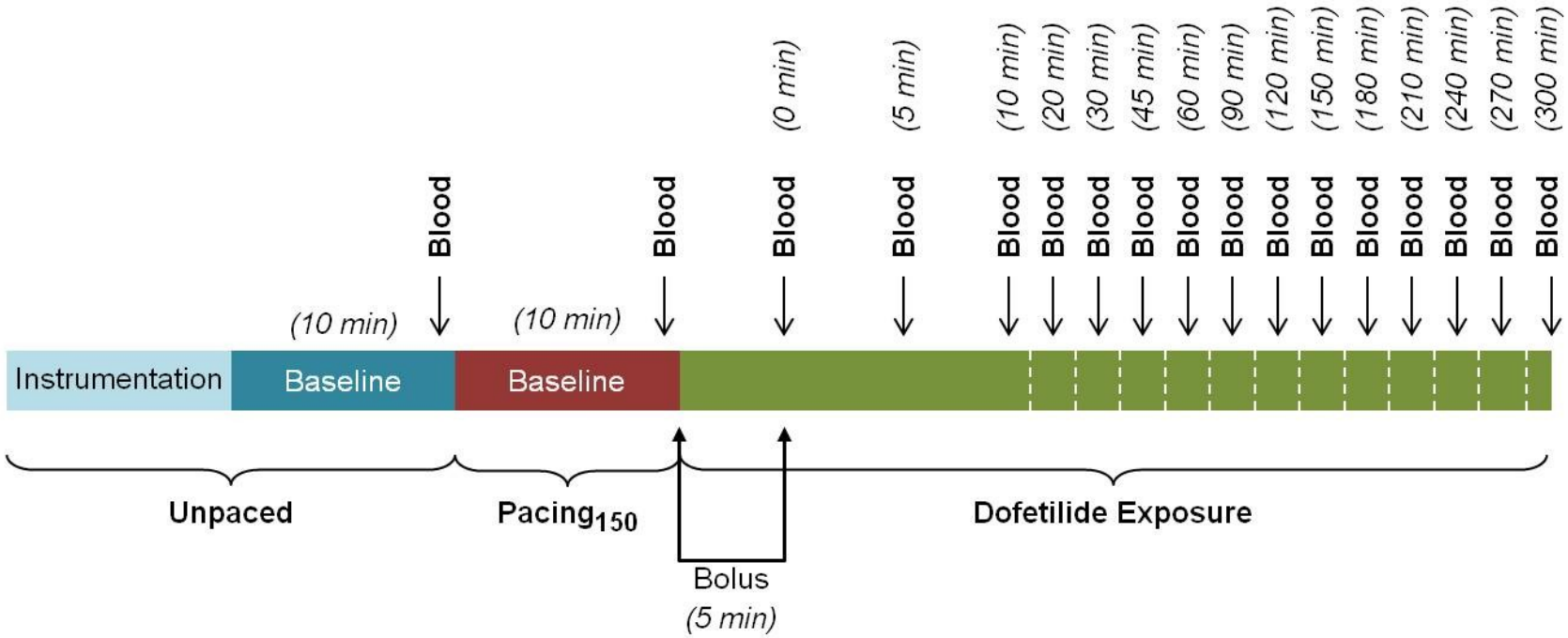


Figure 4.1 Study Design including the schedule for blood harvesting relative to pacing initiation.

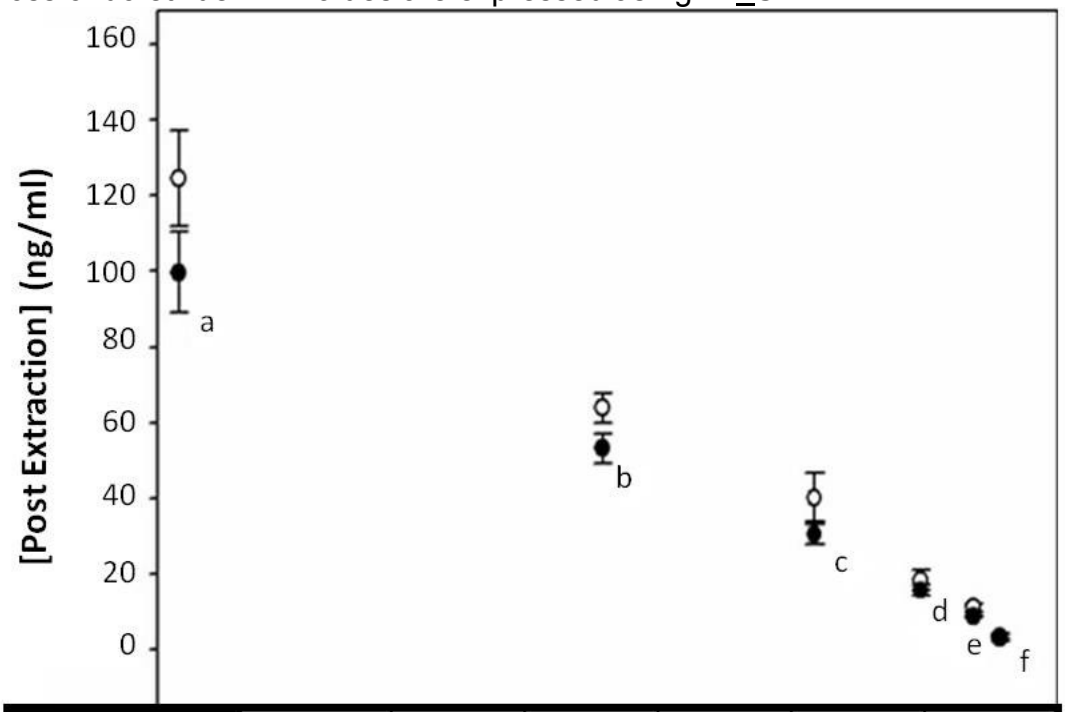


DATA

DOFETILIDE EFFICIENCY ASSESSMENT

Dofetilide concentrations were determined by high pressure liquid chromatography following a liquid-liquid ether extraction. The efficiency of the extraction is summarized in Figure 4.2 for the peak height and area under the chromatographic curve for dofetilide (n=5). The lowest detectable standard was determined to be the limit of detection for this procedure (7.6ng/ml). Measurements from area under the curve had a higher recovery (56.9%) versus peak height (49.0%) and therefore, area was used for kinetics calculations.

Figure 4.2 Extraction efficiency experiments (n=5) from serum using dofetilide naive serum spiked with a known amount of dofetilide prior to extraction and measured using HPLC from peak height (closed circle) and area under the curve (open circle) with corresponding actual and percent loss of dofetilide. All values are expressed as ng/ml±SEM.



		$[Dof]_{pre}$	244_a	122_b	61_c	30.5_d	15.3_e	7.6_f
$[Dof]_{post}$	Area (±)		124.4 (± 12.6)	63.8 (± 3.8)	40.2 (±6.5)	18.4 (± 2.6)	11.1 (± 1.1)	3.0 (± 0.8)
	Height (±)		99.7 (± 10.8)	53.2 (± 3.8)	30.5 (± 2.7)	15.7 (± 1.4)	8.8 (± 0.2)	3.4 (± 0.7)
Actual Loss	Area		119.6	58.2	20.8	12.1	4.2	4.6
	Height		144.3	68.8	30.5	14.8	6.5	4.2
Percent Loss	Area		49.0	47.7	34.1	39.7	27.5	60.5
	Height		59.1	56.4	50.0	48.5	42.5	55.3

BASELINE CHARACTERISTICS

Hemodynamic and electrocardiographic parameters for the period just prior, and just after beginning of pacing are summarized in Table 4.0. The prepacing heart rate was significantly increased (24.9±7.8 beats/min) to 150 beats/min. A corresponding and statistically significant reduction in QT interval (23.6±10.5msec), T_{peak} to T_{end} segment (1.5±1.6msec) were observed. The QRS duration, peak systolic pressure and *d*P/*dt* were not significantly altered.

Table 4.0 Summarized values for measured baseline characteristics including heart rate (beats/min), QT interval (msec), peak systolic (mmHg), left ventricular pressure (LVP), and *d*P/*dt* from the LVP (mmHg/sec) collected before and during high right atrial pacing at a rate of 150 beats per minute in anesthetized domestic pigs (n=7). *p<0.05

	Unpaced Parameters		**Paced Parameters**		**P-value**
	Average	**SEM**	**Average**	**SEM**	
Heart Rate (BPM)	125.1	7.8	150	0	0.02*
QT Interval (msec)	254.3	11.9	230.7	9.1	0.02*
TpTe (msec)	38.9	1.9	37.4	1.4	0.03*
QRS Duration (msec)	42.1	1.8	42.0	2.0	0.59
Systolic Pressure (mmHg)	87.1	2.7	91.8	4.2	0.20
*d*P/*d*t (mmHg/sec)	1241.5	76.1	1292.7	93.7	0.54

PHARMACOKINETICS AND REPOLARIZATION DYNAMICS OF DOFETILIDE

The time course for changing serum concentrations following intravenous dofetilide (0.2mg/kg) are shown in Figure 4.3a. The data acquired from the chromatographic area had a consistently higher concentration at each sampling point. The kinetic area under the curve for the concentration – time relationship from the chromatographic area was 141.15ng/ml and the maximum concentration was 69.04ng/ml. A dose-response relationship for the change in QT interval, and serum dofetilide concentration is shown in Figure 4.3b.

Serum dofetilide concentrations were plotted against time after dosing in semi-log fashion to evaluate the elimination pattern and estimate elimination rate constant(s). These data are depicted in Figure 4.4 and summarized in Table 4.1. A single linear relationship did not adequately fit the data indicating two apparent rate constants, K_e1 and K_e2. The pattern of elimination deviated from the typical two-compartment model since the earlier rate constant (K_e1) was substantially less than the later rate constant (K_e2). This suggests that the faster K_e2 elimination process exhibits saturation at a concentration of 48.2 ± 11.1ng/ml. These elimination characteristics for dofetilide in pigs differ from that described for other species (Table 4.2).

Figure 4.3 [a] Dofetilide concentration summary (n=7) with corresponding representative electrocardiographic morphology alterations over 5 hours from a single intravenous dofetilide infusion (t_0). [b] Dofetilide – QT interval response curve, measured as change from baseline, for animals (n=7) administered a single (200mcg/kg) bolus of dofetilide while paced continually at 150 beats/min.

Figure 4.3 (Cont'd)

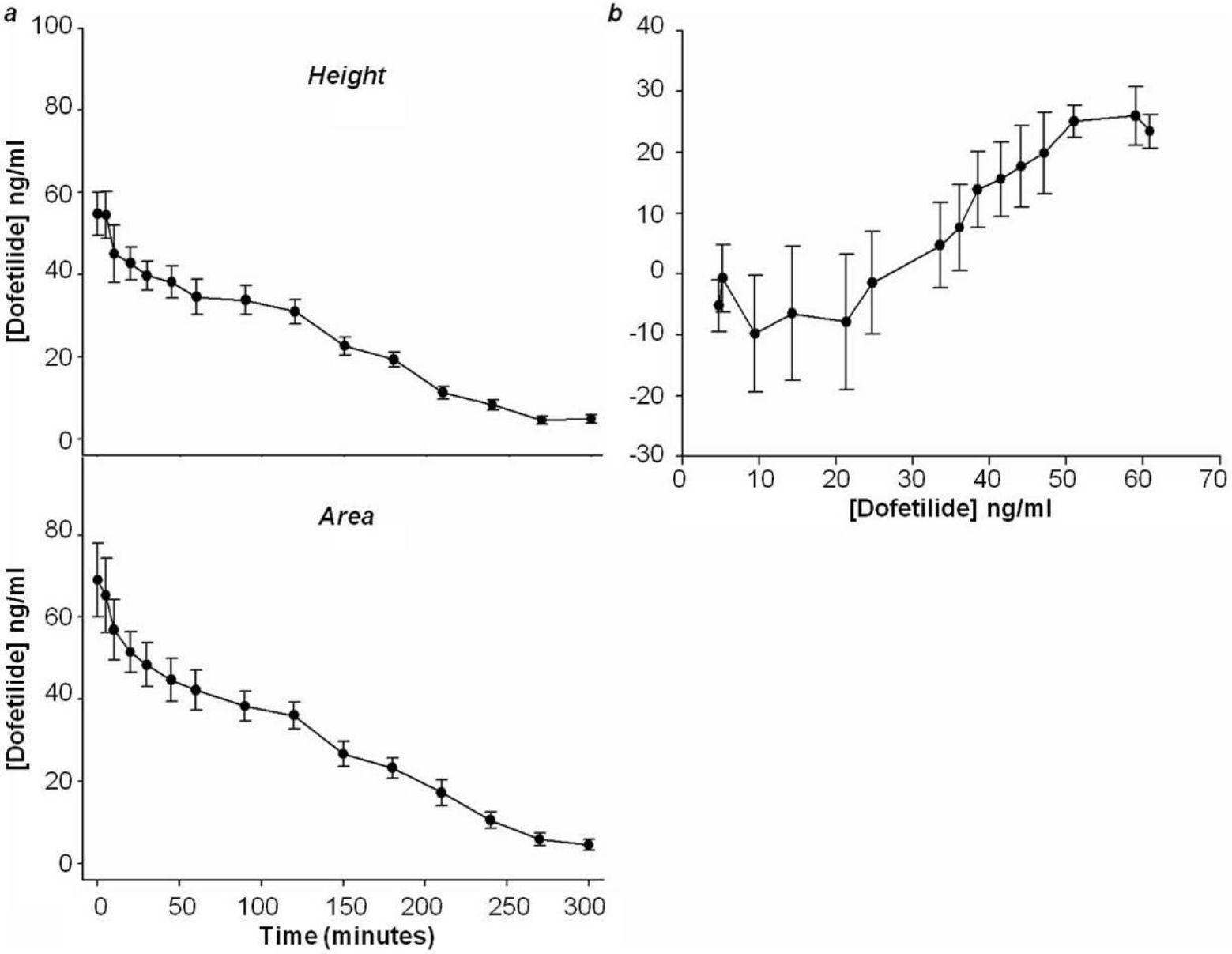


Figure 4.4 Illustration of the group (n=7) dual-effect pharmacokinetic response following dofetilide administration.

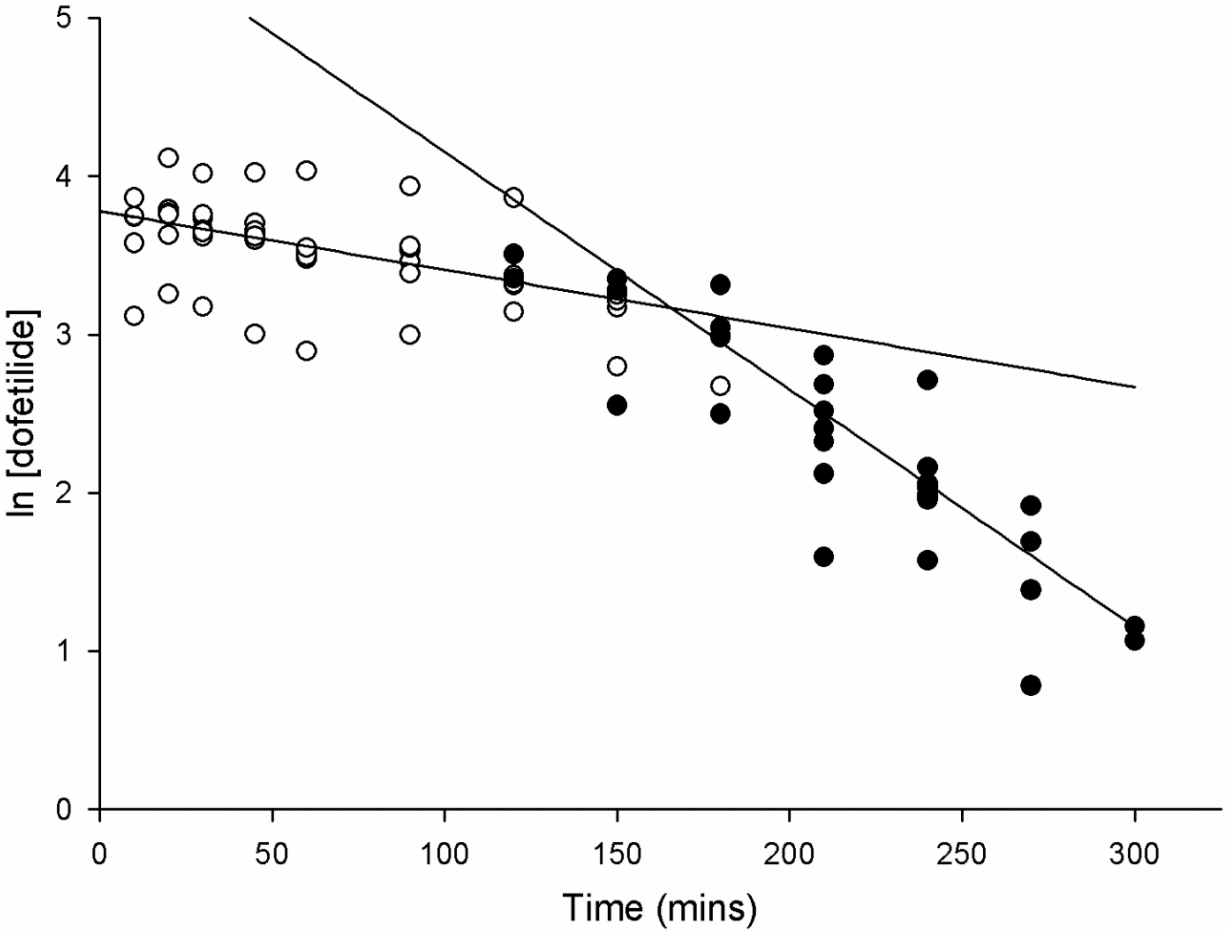


Table 4.1 Summary of the group (n=7) dual-effect pharmacokinetic data used to derive linear regressions of a dual-effect system. $\ln(C_{max})$ ng/ml; value (min^{-1}); V_{dist} = volume of distribution; C_{max} L/kg (maximal measured concentration); C_{extr} L/kg (curve extrapolation to time zero).

		K_e1			K_e2			V_{dist}	
	$\ln(C_{max})$	y-int	value	R^2	y-int	value	R^2	C_{max}	C_{extr}
Avg	3.98	3.78	-0.0037	0.88	5.65	-0.0150	0.92	2.03	2.51
StDev	0.26	0.32	0.0010	0.10	1.20	0.0059	0.09	0.58	0.86
Coef Var %	6.6	8.4	26.5	10.9	21.3	39.6	10.1	28.6	34.3

Table 4.2 Summary of resultant parameters from this study, and the comparison of those to similar studies conducted in other species. In the present study, QT interval was corrected using Bazett's formula for the purposes of comparison.

Species	Dose (mg/kg)	C max (ng/ml)	AUC (ng/ml)	Max change in QTc (ms)	References
Swine	0.2	69.04	141.15	57	Present Study
Dog	0.3	60.15	261.89	64	Haushalter et al. (2008)
Monkey	0.03	1.85	6.37	50	Haushalter et al. (2008)
Human	0.04	3.55	18.89	19	Allen et al. (2000)

ELECTROCARDIOGRAPHIC AND HEMODYNAMICS EFFECTS OF DOFETILIDE

The QT interval, compared to baseline (n=3), reached its maximal within 10 minutes after completion of the dofetilide infusion. The QT interval steadily returned to control levels at 175 minutes after dosing (Figure 4.5; Table 4.3). Unlike the slowly declining QT interval, peak left ventricular systolic pressure and *d*P/*dt* increased steadily after dosing, both reached a maximum 220 minutes post dosing with a maximal change in peak left ventricular pressure of 10.5mmHg, and left ventricular *d*P/*d*t of 310mmHg/min.

Figure 4.5 Summary of resulting QT interval, maximum systolic pressure from LVP ($maxSP_{LVP}$) and the positive *d*P/*dt* following dofetilide infusion (t_0). The values are normalized to the animals individual baseline parameters (n=7) and compared to controls (n=3) paced at 150 beats/min. Open triangles are the data from control (no dofetilide); filled squares represent the dofetilide treated animals.

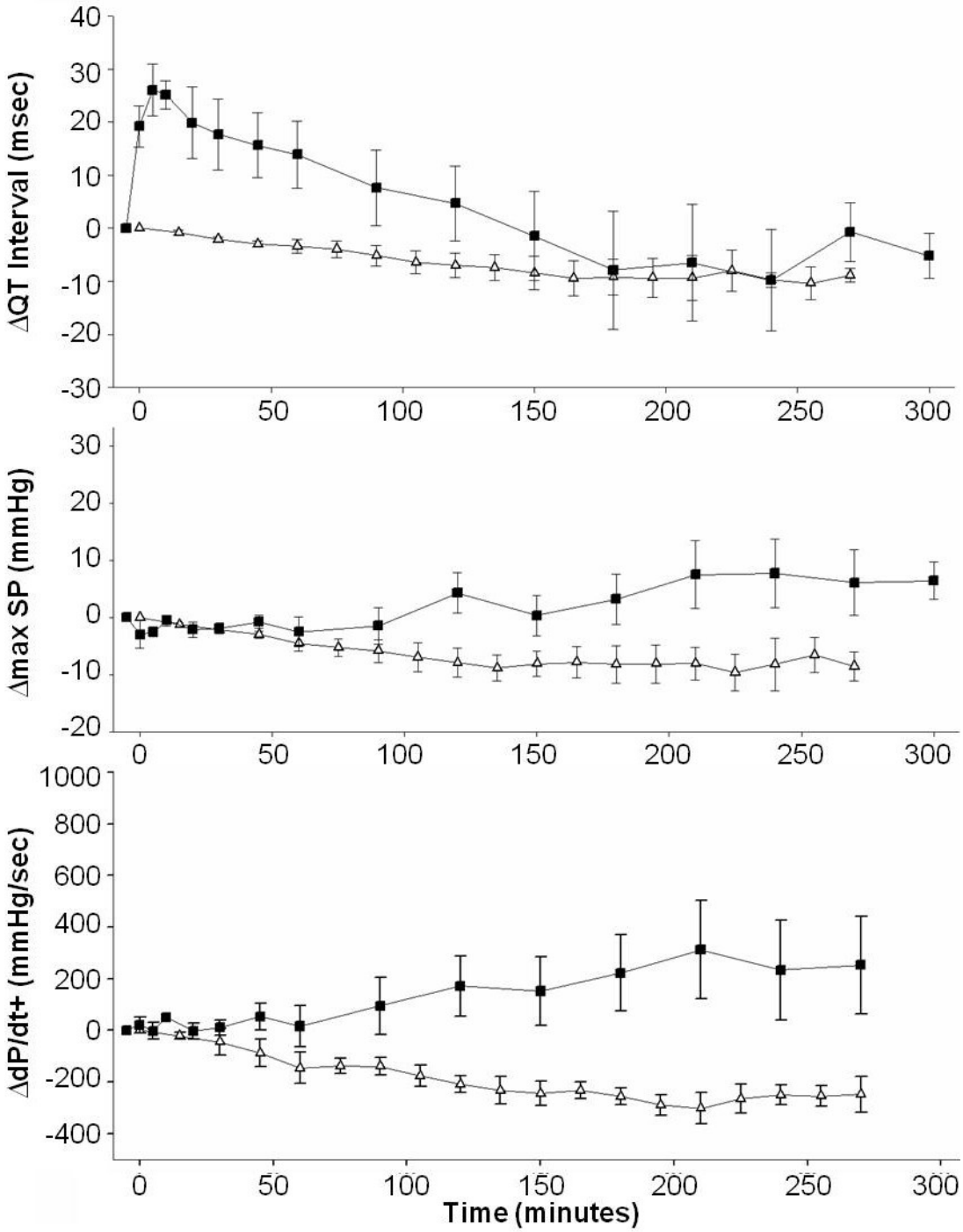


Table 4.3 Summary of resulting QT interval, maximum systolic pressure from LVP ($maxSP_{LVP}$) and the positive *d*P/*dt* following dofetilide infusion (t_0). The values are normalized to the animals individual baseline parameters (n=7) and compared to controls (n=3) paced at 150 beats/min.

Time	ΔQTI				Δmax SP_{LVP}				Δ*dP/dt*			
	Dofetilide		Vehicle		Dofetilide		Vehicle		Dofetilide		Vehicle	
	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM	AVG	SEM
0	19.2	3.9	0.0	0.0	-3.0	2.4	0.0	0.0	21.2	30.4	0.0	0.0
5	26.0	4.8			-2.6	0.9			-2.4	33.1		
10	25.1	2.6			-0.6	0.9			50.1	0.0		
15			-0.8	0.4			-1.3	0.3			-21.4	13.0
20	19.9	6.7			-2.1	1.3			-2.2	31.1		
30	17.7	6.7	-2.0	0.4	-1.8	0.6	-2.1	0.7	11.1	29.5	-44.6	50.8
45	15.6	6.1	-3.0	0.5	-0.8	1.1	-3.0	0.6	53.3	50.5	-88.3	53.5
60	13.9	6.3	-3.3	1.3	-2.5	2.6	-4.5	1.3	16.0	78.6	-146.0	61.0
75			-4.0	1.6			-5.3	1.5			-137.8	30.0
90	7.6	7.1	-5.1	2.0	-1.5	3.2	-5.9	2.0	94.8	111.1	-139.3	32.9
105			-6.4	2.1			-6.9	2.5			-177.0	41.4
120	4.7	7.0	-7.0	2.3	4.3	3.6	-7.9	2.5	171.8	116.7	-208.7	32.6
135			-7.4	2.5			-8.8	2.2			-232.3	52.0
150	-1.5	8.4	-8.4	3.2	0.3	3.5	-8.1	2.2	151.9	133.4	-244.0	47.2
165			-9.4	3.3			-7.8	2.8			-232.0	32.0
180	-7.9	11.1	-9.2	3.4	3.2	4.4	-8.2	3.2	222.5	148.3	-256.2	32.9
195			-9.4	3.6			-8.1	3.3			-288.8	39.5
210	-6.5	11.0	-9.4	4.2	7.5	5.9	-8.1	2.9	312.1	190.7	-301.8	59.5
225			-8.0	3.9			-9.6	3.2			-265.5	56.7
240	-9.8	9.6	-9.8	1.4	7.7	6.0	-8.2	4.6	233.7	193.2	-249.6	38.6
255							-6.5	3.1			-254.7	39.7
270	-0.7	5.5	-10.4	3.1	6.0	5.7	-8.5	2.5	252.6	188.3	-247.4	69.9
300	-5.2	4.2	-8.8	1.3	6.4	3.3						

OVERALL CONCLUSION

Dofetilide, when administered to pigs, has a dual-effect elimination that is unusual and previously unreported. Dofetilide induces a dose dependent increase in QT interval, peak LV pressure, and *d*P/*dt*. When expressed as the ratio of change in QT interval to CMax, pigs are similar to dogs, but substantially less sensitive to dofetilide than monkeys or humans.

CHAPTER FIVE – THE EFFECT OF TACHYCARDIC IRREGULARITY ON THE RECOVERY OF REPOLARIZATION PROLONGATION IN I_{Kr} ANTAGONIZED DOMESTIC PIGS

INTRODUCTION

It is well established that certain antiarrhythmic drugs have a substantial risk of RRA of one percent or more. Drugs such as quinidine, sotalol, ibutilide and dofetilide are all examples of drugs that enhance RRA risk, especially when combined with cardiovascular disease such as atrial fibrillation (Soyka et al., 1990; Torp-Pedersen et al., 1999; Coplen et al., 1990; Roden et al., 1986; Stambler et al., 1996). Atrial fibrillation is the current primary indication, and a large percentage are receiving, antiarrhythmic drug therapy (Darbar & Roden, 2006). Dofetilide, compared to other agents, is a specific antagonist for the I_{Kr}: the currently implicated mechanism for ~~in~~ nearly all forms of RRA liability. It also has strong reverse-use dependence, meaning that the drugs pharmacodynamics action is facilitated at lower heart rates, yet restricted at higher rates. The mechanism responsible for this with dofetilide is not completely understood but is shared by other drugs with similar I_{Kr} effects such as E4031 and sotalol.

A clinical observation over the past 25 years has been that RRAs often occur in patients with atrial fibrillation after conversion to sinus rhythm (McCray, et al., 2006). This could logically represent either a protection from RRA during atrial fibrillation or vulnerability

toward RRA after conversion to sinus rhythm. Because atrial fibrillation patients receive antiarrhythmic drug therapy and have a heightened risk for RRA, atrial fibrillation itself might exert a poorly understood influence on the QT interval both during the arrhythmia or shortly after its conversion to sinus rhythm. Atrial fibrillation is distinct, in part due to its irregular heart rate. Understanding mechanisms underlying atrial fibrillation repolarization effects would be an important step forward in understanding the increase risk for RRA when an I_{Kr} antagonist is used.

Having evaluated the repolarization response of fixed, sinusoidal and random paced normal domestic pigs and finding there to be no significant alteration of repolarization indices, the focus is shifted to a study of the effect of sinusoidal and random pacing in domestic pigs treated with dofetilide. This study is designed to test the hypothesis that I_{Kr} antagonist treatment with dofetilide alters repolarization dynamics in the presence of irregular tachypacing differently than its effects during sine wave tachypacing.

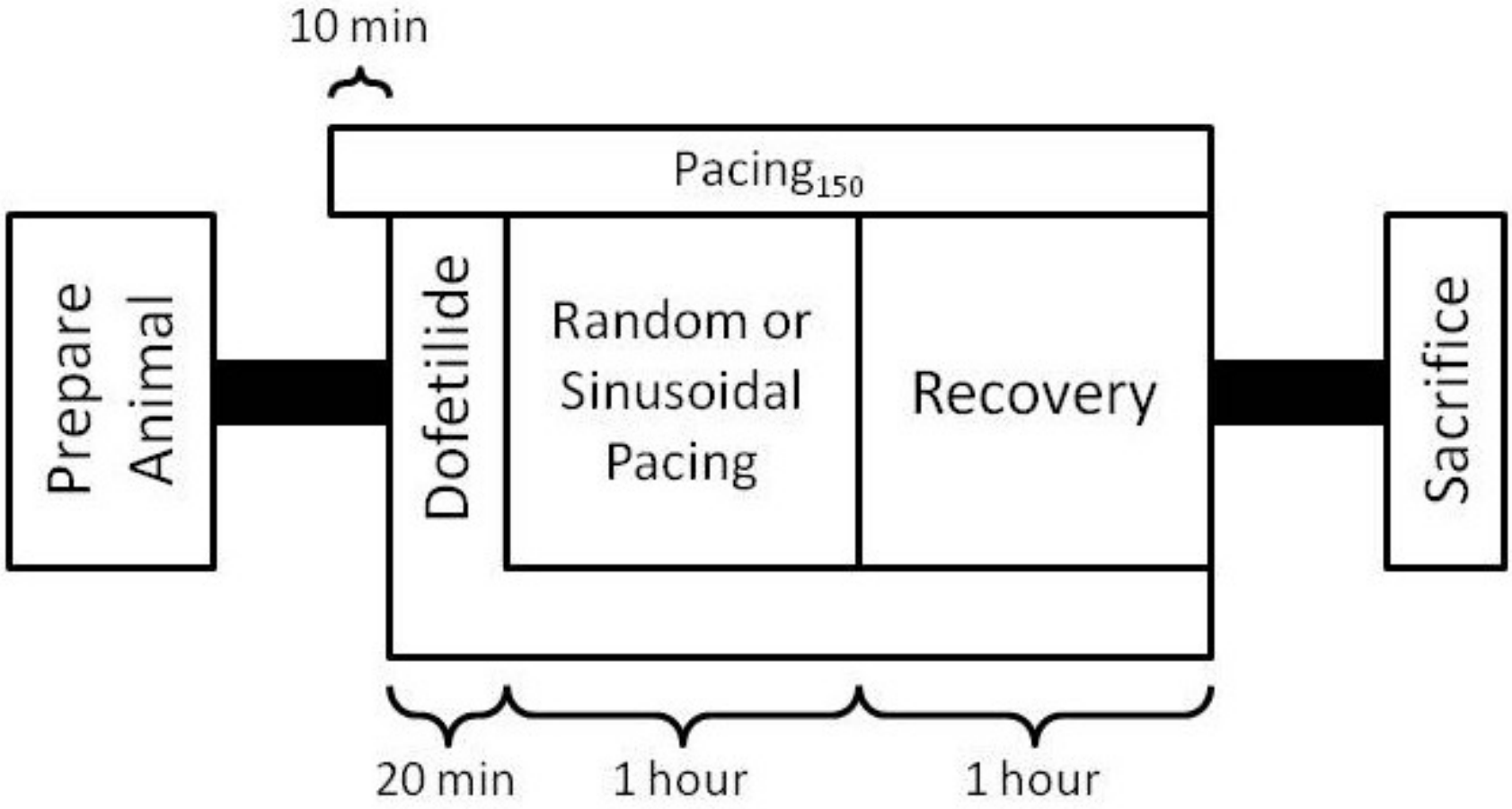
SPECIFIC METHODS

Domestic pigs (n=7) were anesthetized with isoflurane and instrumented as previously described. The basic protocol is outlined in Figure 5.0. Baseline data was collected for at least 15 minutes without pacing and continued after initiating high right atrial pacing at 150 beats per minute. After 10 minutes of pacing at 150 beats/min, animals were then administered, a 5-minute infusion of dofetilide (Sigma-Aldrich, St. Louis, MO)

through a peripheral ear vein, at a dose of 50mcg/kg. This loading infusion was followed by a continuous rate infusion of 0.35mcg/kg/min. The dose for dofetilide administration was based on the pharmacokinetic results summaries in Chapter 4 of this dissertation. The experimental intent was to study QT interval dynamics while serum concentrations of dofetilide are relatively constant. The nominal goal was a deviation of 15% or less from the 10-minute serum concentration. It was calculated based on the average K_e1 (-0.0037 min^{-1}), which represents the slower, high-capacity elimination. This does was selected because a) it is similar to doses in the literature given to pigs and dogs b) it generates a detectable plasma concentration of dofetilide, and c) based on previous work QT interval was predicted to increase by approx 20-30 msec while still maintaining a 1:1 AV conduction rate at high atrial pacing rates. The continuous infusion rate was calculated with the assumption of a one-compartment model. This was justified because the desired serum concentration exceeded the saturation for K_e2.

Sequential 8ml blood draws for serum collection were performed according to the outline in Figure 4.1. After 300 minutes the pacing was terminated and an additional 15 minutes of data was collected.

Figure 5.0 Protocol to assess repolarization alteration following domestic pigs fixed paced at 150 beats/min ($Pacing_{150}$) and randomized to either sinusoidal or random tachypacing.

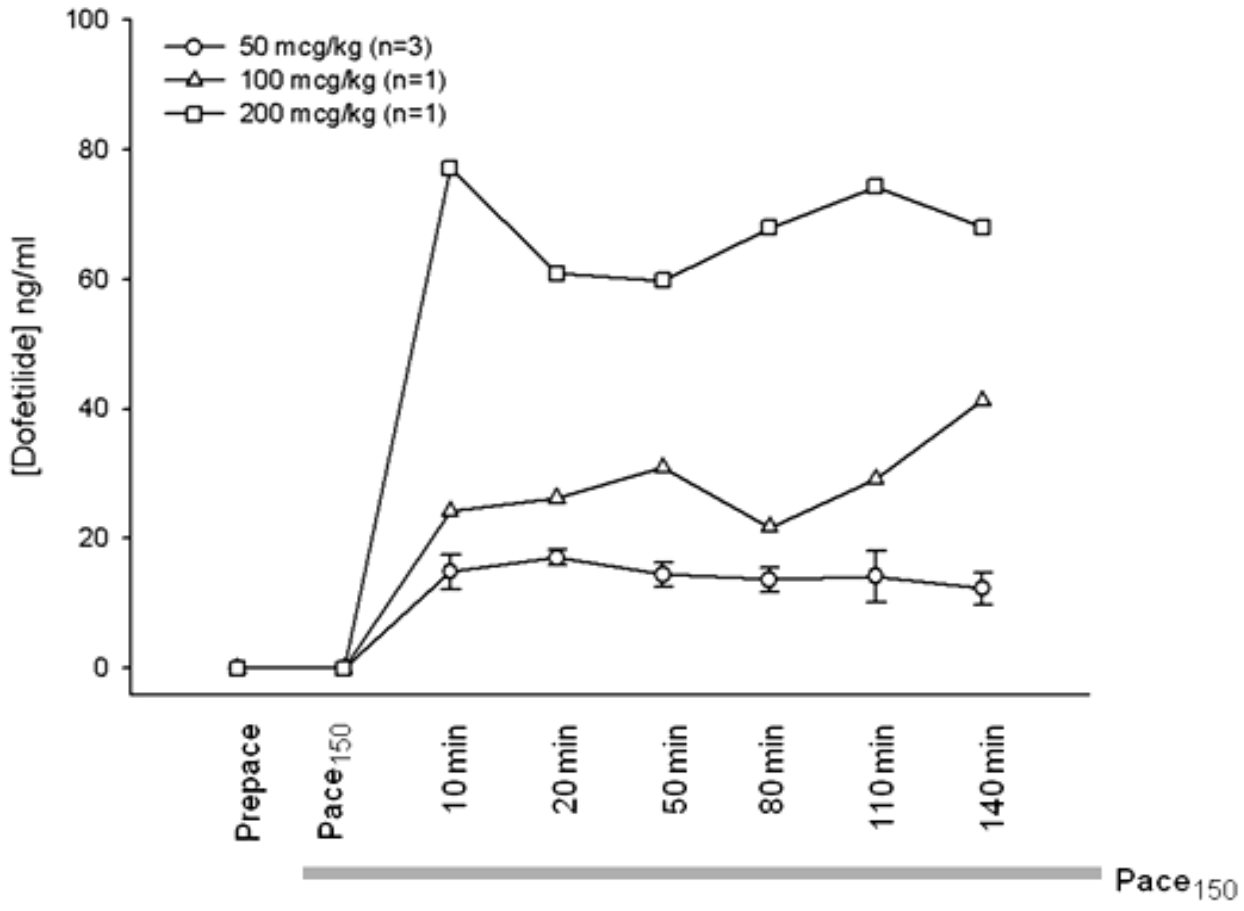


DATA

DOFETILIDE EXPOSURE WHILE PACING

To test the hypothesis that I_{Kr} antagonist treatment with dofetilide alters repolarization dynamics in the presence of irregular tachypacing differently than its effects during sine wave tachypacing, a relatively constant effect of this drug throughout the tachypacing period is needed. There is limited data in the literature relating a dose of dofetilide that alters repolarization parameters in domestic pigs. However, there are many citations validating a short-term dose of dofetilide in the context of other species. Figure 5.1 is the data corresponding to serum concentrations of dofetilide given at doses of 200mcg/kg, 100mcg/kg, and 50mcg/kg as a bolus (administered over 5 minutes) and followed immediately by a constant rate infusion of 0.35mcg/kg/min. Each of these was evaluated before (Pre-pace) and after pacing at 150 beats/min ($Pace_{150}$). The dose of 50mcg/kg was selected for further study because similar doses were given in the literature to dogs, it generates a detectable serum concentrations and the QT interval prolongs to a degree of 20-30msec.

Figure 5.1 Dofetilide exposures in domestic pigs paced at 150beats/min and given a bolus of dofetilide (200, 100 or 50mcg/kg) followed by a continuous infusion (0.35mcg/kg/min) for 140 minutes. [The text in this figure is not meant to be decipherable, but for visual reference only.]



EVALUATION OF THE EFFECT OF TACHYPACING ON THE QT INTERVAL IN PIGS TREATED WITH DOFETILIDE

The instantaneous heart rate for tachypacing was adjusted in this arm of the study to prevent a loss of 1:1 AV nodal conduction. This was necessary because dofetilide can depress AV nodal conduction. The average heart rate for sinusoidal and random pacing was set to 180beats/min (CL = 333msec), and the control pacing was performed at the lowest heart rate pacing rate. The range of heart rates was retained and set to 60 beats/min as outlined in Figure 5.2.

Pigs in the control group were fixed-paced at 150 beats/min ($Pace_{150}$) throughout the experiment (Figure 5.3). For the sinusoidal and random groups, pacing at 150beats/min was discontinued (at time -60) and tachypacing was initiated for one hour. After the termination of tachypacing, pacing at 150 beats/min was resumed. After cessation of tachypacing, the QT interval was evaluated every 5 minutes for 30 minutes, and then at the end of one hour for 5 additional minutes. The QT interval for the control group increased shortly after initiating dofetilide but steadily declined for the remainder of the data collection period despite relatively constant serum dofetilide concentrations. This control response resembles what would be seen with tachyphylaxis. In contrast, QT interval for sinusoidal and random tachypacing declined during tachypacing as expected, but then recovered to the pre-tachypacing level. Neither tachypacing group exhibited any semblance of tachypacing, even one hour after tachypacing cessation. Irregular tachypacing did not differentially affect repolarization compared to sinusoidal

tachypacing. The experimental hypothesis was therefore not sustained. However, the results did suggest a possible mechanism for post tachypacing RRA vulnerability.

Figure 5.2 Diagrammatic representation of high right atrial pacing paradigms (CL_{avg}:333) sinusoidal (green) and random (pink) tachypacing.

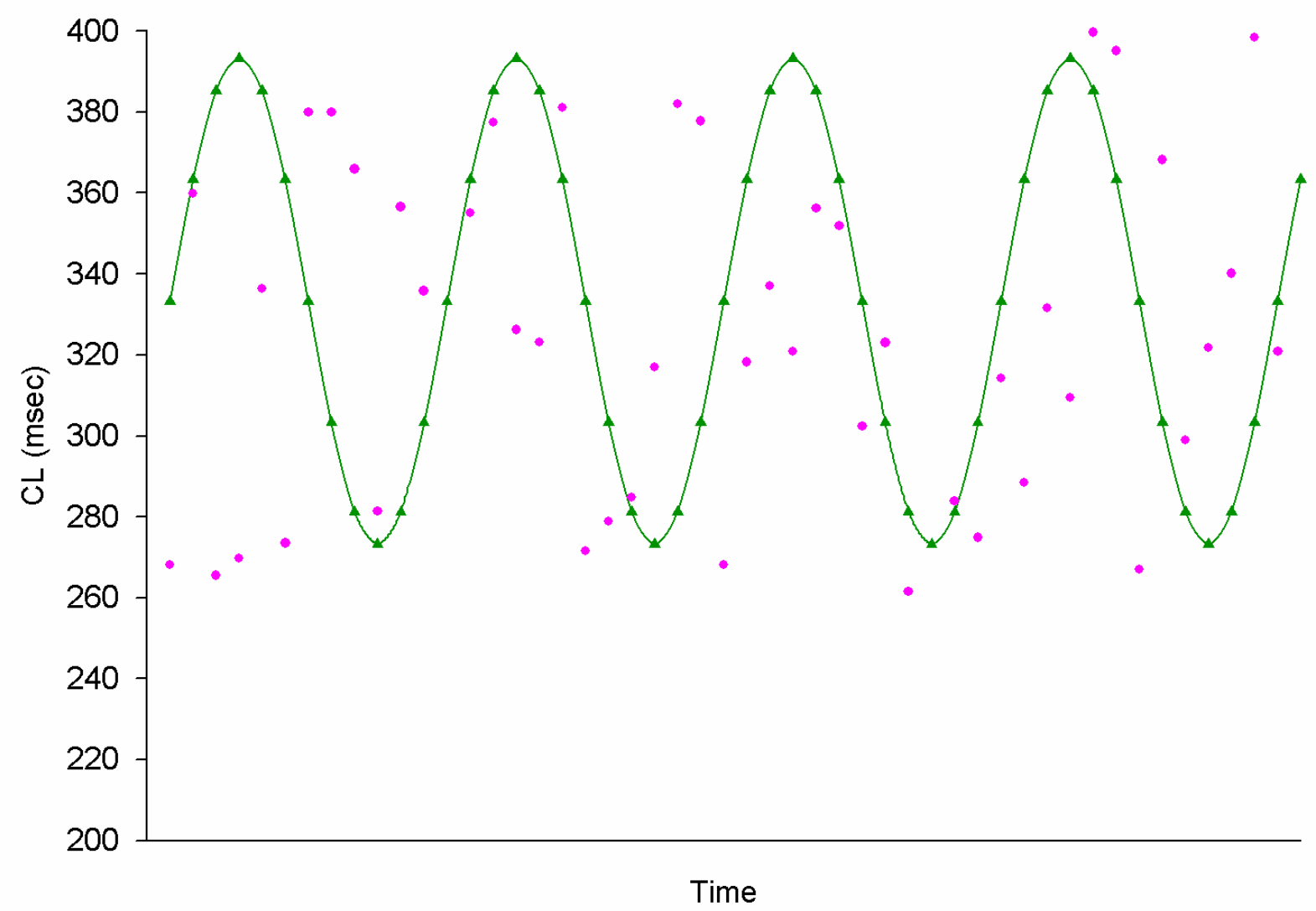
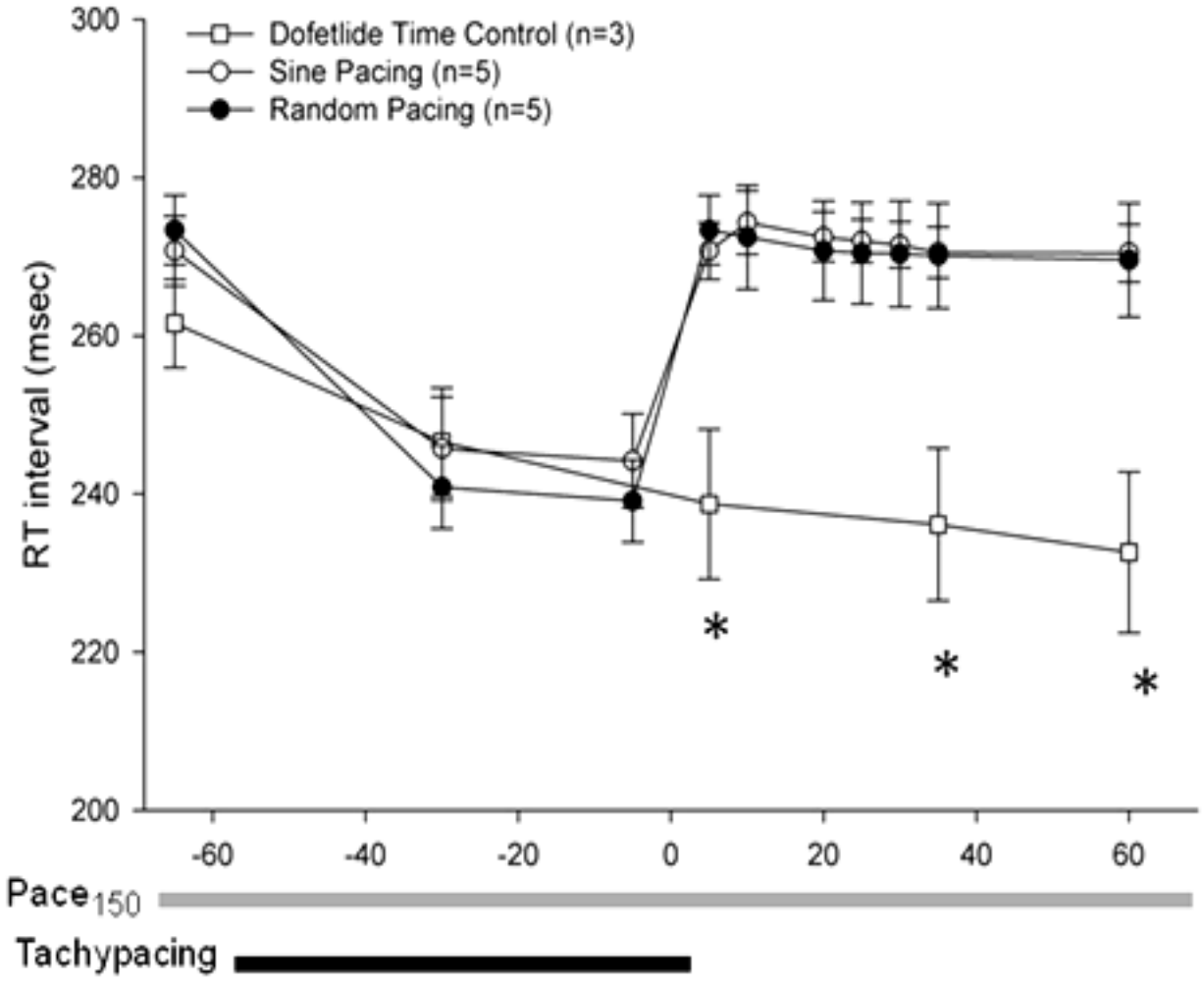


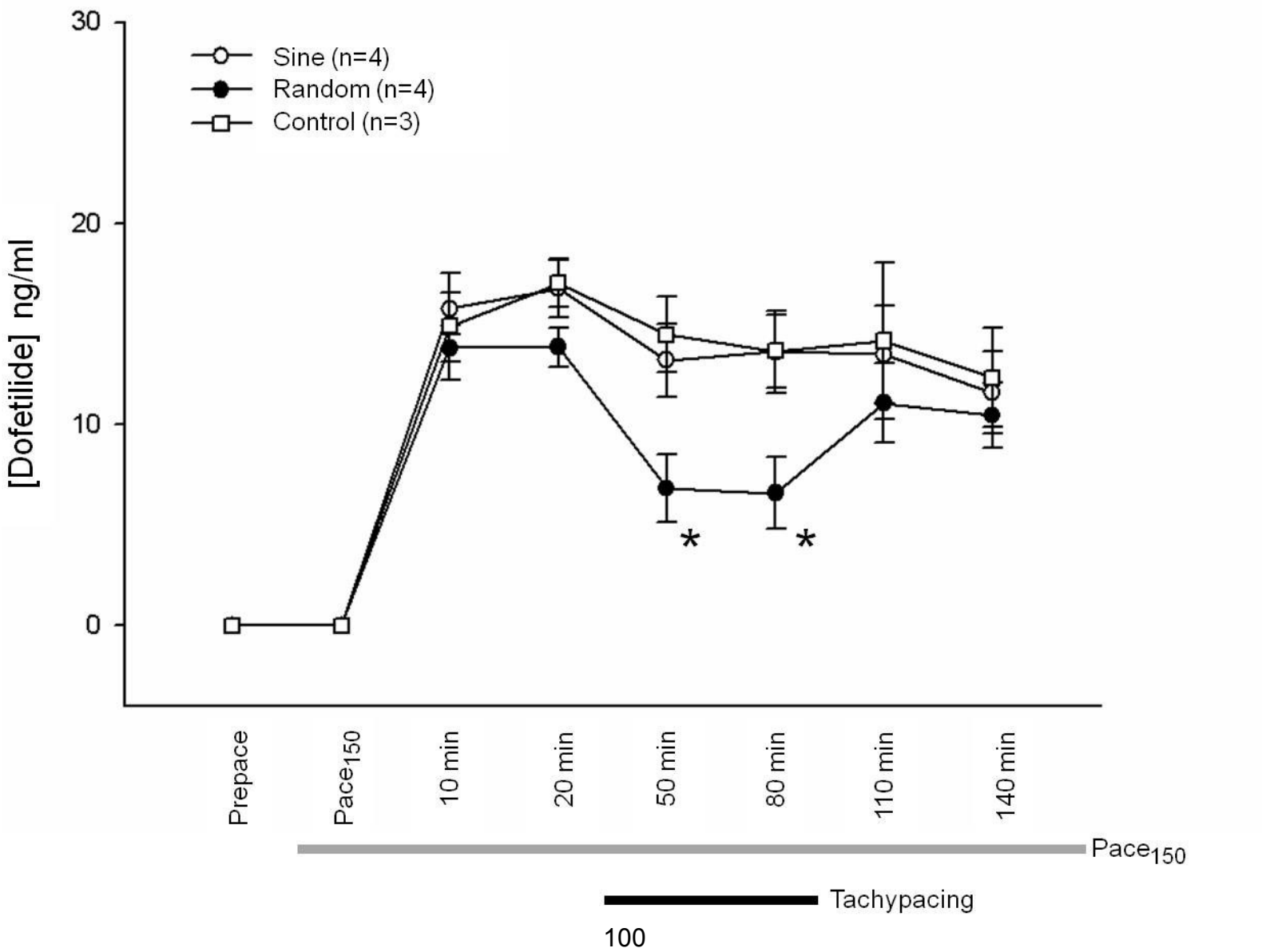
Figure 5.3 QT (RT) assessment of dofetilide treated domestic pigs paced at 150 beats/min for the entire experiment (Dofetilide Time Control) or with pacing interrupted by one hour of sinusoidal (Sine) or Random tachypacing. (*p=0.042)



DOFETILIDE EXPOSURE WHILE TACHYPACING

To evaluate the exposure of dofetilide during experimental tachypacing, serum dofetilide was measured before and after sinusoidal and random tachypacing and compared to control. Outside of the time that tachypacing occurred, pacing was performed at 150 beats/min ($Pace_{150}$). During periods corresponding to sinusoidal and random tachypacing, there was a significant reduction in serum dofetilide concentrations in the random tachypacing group ($p=0.016$). That change recovered following the termination of tachypacing ($p=0.022$) (Figure 5.2).

Figure 5.4 Dofetilide exposures in domestic pigs exposed to dofetilide and paced at 150beats/min and treated with either Control pacing (150 beats/min) for the entire experiment or with pacing interrupted by one hour of sinusoidal (Sine) or Random tachypacing. (*p<0.05)



EVALUATION OF THE EFFECT OF TACHYPACING ON T_PT_E IN PIGS TREATED WITH DOFETILIDE

The T_pT_e was evaluated as a surrogate for transmural dispersion of repolarization. Pigs were fixed-paced at 150beats/min ($Pace_{150}$) throughout the experiment (Figure 5.5). For the sinusoidal and random groups, pacing at 150beats/min was discontinued (at time -60) and tachypacing was initiated for one hour. There was no significant change in T_pT_e throughout the evaluation period.

Figure 5.5 Transmural dispersion of repolarization assessment (T_pT_e) of dofetilide treated domestic pigs paced at 150 beats/min for the entire experiment (Dofetilide Time Control) or with pacing interrupted by one hour of sinusoidal (Sine) or Random tachypacing.

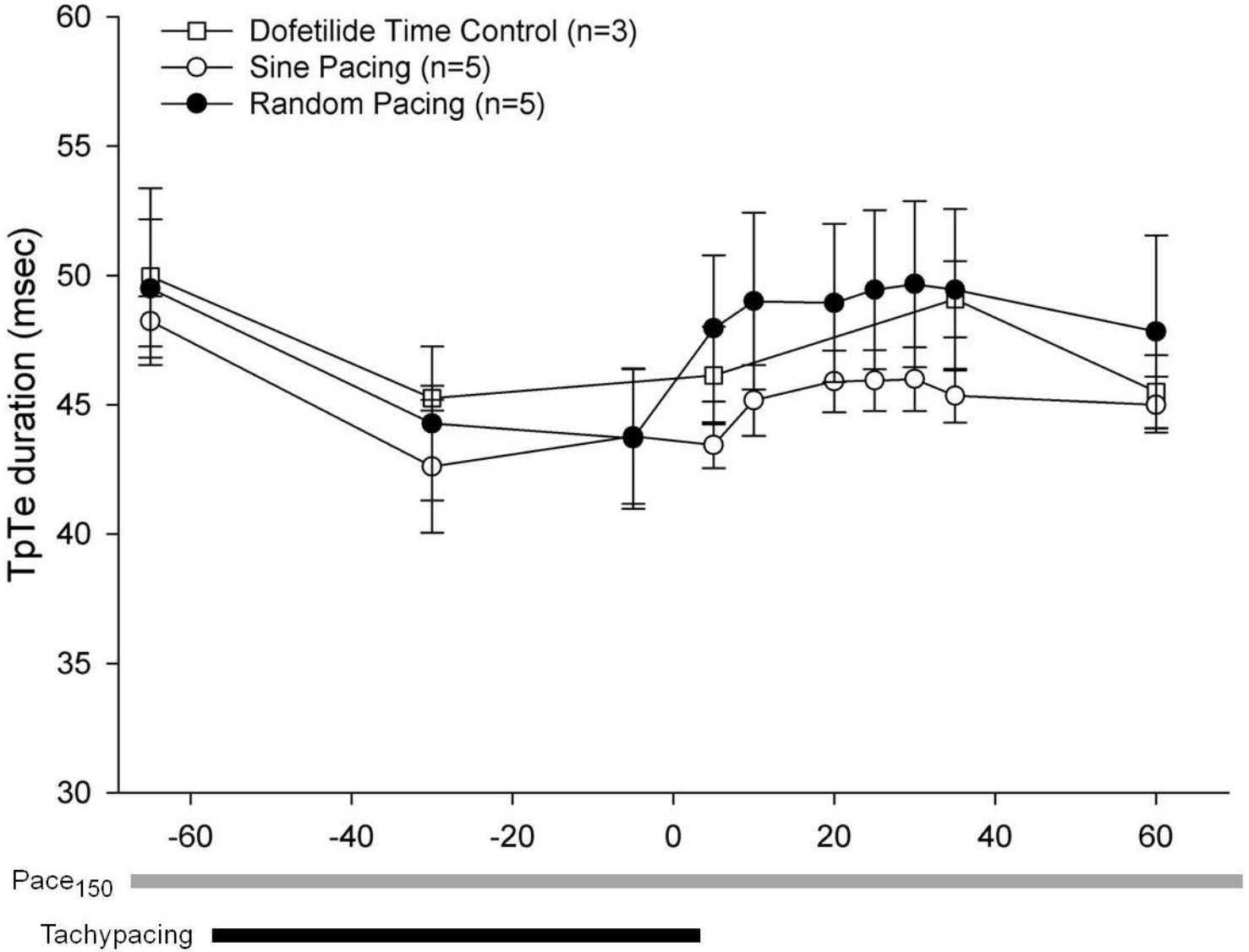


Table 5.0 QT and TpTe (TpTe) summary of dofetilide treated domestic pigs paced at 150 beats/min for the entire experiment (Control) or with pacing interrupted by one hour of sinusoidal (Sine) or Random tachypacing.

	Rel Time	-60	-30	-5	0	5	10	15	20	25	60
QTI	Sine RTI	270.7	245.7	244.2	270.7	274.3	272.5	272.0	271.5	270.5	270.4
	Sine SEM	4.4	6.5	5.9	3.6	4.0	3.1	2.7	2.9	3.2	3.6
	Random RTI	273.4	240.9	239.1	273.4	272.5	270.8	270.5	270.4	270.1	269.6
	Random SEM	4.4	5.3	5.2	4.4	6.6	6.3	6.4	6.7	6.7	7.2
	Control RTI	261.6	246.6		238.7					236.1	232.6
	Control SEM	5.6	6.8		9.5					9.6	10.2
	Rel Time	-60	-30	-5	0	5	10	15	20	25	60
TpTe	Sine TpTe	48.2	42.6	43.8	43.4	45.2	45.9	45.9	46.0	45.3	45.0
	Sine SEM	1.0	2.6	2.6	0.9	1.4	1.2	1.2	1.2	1.0	1.1
	Random TpTe	49.5	44.3	43.7	48.0	49.0	48.9	49.5	49.7	49.5	47.8
	Random SEM	2.7	3.0	2.7	2.8	3.4	3.1	3.1	3.2	3.1	3.7
	Control TpTe	50.0	45.3		46.1					49.1	45.5
	Control SEM	3.4	0.5		1.9					1.5	1.4

OVERALL CONCLUSION

Dofetilide, an antiarrhythmic that is also proarrhythmic, has a lower serum concentration during random tachypacing compared to sinusoidal tachypacing, suggesting that the clinical theory of protection may be due to a pharmacokinetic protective effect during atrial fibrillation. Random and sinusoidal tachypacing in dofetilide treated pigs is associated with an increase in the QT interval just after the termination of tachypacing to levels indistinguishable from pre-tachypacing levels. This suggests that tachycardia of any type may confer a post-tachycardia vulnerability due to a fast, rather than an irregular, heart rate.

CHAPTER SIX - DISCUSSION

SHORT-TERM IRREGULAR TACHYPACING FAILS TO DIFFERENTIALLY ALTER COMMONLY USED REPOLARIZATION PARAMETERS WHEN COMPARED TO REGULARLY VARYING, OR FIXED TACHYPACING

The results of this dissertation do not support the hypothesis that a putative protection effect during atrial fibrillation is provided by changes in QT interval or QT interval dynamics. During tachypacing, the QT interval shortened; consistent with its expected rate dependency. There was no difference between sinusoidal and random tachypacing for QT interval, T_pT_e or QT variability. Fixed tachypacing was included in this arm of the study to mimic a fixed-rate tachycardia such as a rapid supraventricular arrhythmia. The rate was set to be the average rate of sinusoidal and random pacing. The QT interval and T_pT_e evaluation between fixed tachypacing and sinusoidal tachypacing was not different.

There are several possibilities for these findings including some technical limitations of the study design and execution. These data were evaluated for several minutes at each interval spread to include baseline, during tachypacing, and after the termination of tachypacing. The three time intervals evaluated during tachypacing presented a particular challenge for data analysis. For each beat during tachypacing, there is a corresponding pacemaker induced stimulation spike. This spike has the potential to be

superimposed on the previous T wave, altering the detection accuracy of the peak-of-T, or end-of-T fiducial points. This could diminish the accuracy of the QT interval and T_pT_e analyses. This need to exclude heart beats vulnerable to this problem had to be balanced with the need to maintain successful analysis from a large and representative sample of beats at each heart interval. The selection of heart beats for analysis was performed by an automated system (EMKA-ECGAuto) that uses user selected ECG models to select its included beats. This automation could conceivably create a selection bias favoring beats with morphology that more closely matches the model beat and inadvertently selects for a subset of all realized heart beat and QT intervals. However, it is impossible to completely eliminate this limitation because the pacing spike occurs with a reproducible stimulation to ventricular excitation coupling interval. Therefore, certain beats will always be impossible to model due to the pacing spike occurring at the same time as the end of the T-wave. This potential problem is greater for rhythms with varying heart beat intervals.

Another consideration is the one hour duration of tachypacing. Is this duration too short for the putative protective effect to emerge? One hour was set as the duration of tachypacing because it reflects a reasonable period based on previous studies examining altered electrophysiology as seen in paroxysmal atrial fibrillation. It is also a reasonable approximation for the total duration of paroxysmal atrial fibrillation. Electrophysiology studies that examined short term pacing (<30 minutes) showed that this can increase cardiac refractoriness by 25 msec and that paroxysmal AF, a condition

that precedes permanent AF, occurs for short periods, encompassing the one hour selection (Daoud et al., 1996).

In addition to time, the heart rate window for tachypacing selected for these experiments might have been unfavorable with regard to detecting RRA protection. The rates selected were higher than those encountered in most humans with atrial fibrillation, but were generally proportional since porcine heart rates tend to be higher than humans. More work would have to be done over a wider range of paced rates to explore this in detail.

Animals selected for most of these studies were female. This was intentionally done because human females represent a population that is more susceptible to RRAs. However, this only is true for humans after sexual maturity (i.e. after puberty). The mechanism for this is not completely understood but in theory it is dependent on sex hormones. Female humans, following sexual maturity, have an average QT interval that is longer than a man. Because these domestic pigs have not attained sexual maturity, it is unlikely that the sex factor is involved in effecting the results.

The disparity between the findings in this study and the clinical reports of humans suggesting RRA protection during atrial fibrillation could be explained by several mechanisms. First, the porcine subjects of this study were a relatively homogeneous and normal group of immature animals which contrasts with most human clinical atrial fibrillation cohorts. Moreover, patient referral bias could unknowingly select for a subset

of humans predisposed to a certain outcome (RRA protection or vulnerability). Finally, the outcome of interest in some clinical studies was RRAs, while in these studies, repolarization surrogates were the primary outcomes. Kaab et al., reported that patients who experienced RRA with QT interval prolonging drugs, developed more QTc lengthening after intravenous sotolol (an I_{Kr} blocking drug), then those in a RRA resistant control group even though both groups had normal baseline QTc durations. (Kaab et al., 2003).

It seems paradoxical that, in the clinic, the achievement and maintenance of sinus rhythm predisposes susceptible patients to the development of RRAs. However, certain characteristics of these human clinical studies may contribute to the data being misinterpreted. In clinical studies, interpretation of results must be considered in light of the subject's characteristics. A heterogeneous study group may cloud conclusions, or may suggest new and interesting lines of research. RRAs are prevalent in patients with structural heart disease, hypokalemia, or receive non-antiarrhythmic drugs with hERG channel blocking effects. These important factors have been proposed as explanations for the protection afforded by atrial fibrillation or the vulnerability after conversion to sinus rhythm. Some patients with a QTc interval within the normal range may respond to drug treatment with excessive lengthening of repolarization and arrhythmias, occasionally leading to sudden cardiac death. In other patients, these drugs do not even prolong cardiac potential duration or only lengthen it moderately. In accordance with these observations, these factors were not studied as part of this dissertation. The

justification for selecting repolarization as a surrogate for RRA, rather than to study RRA susceptibility directly was related to the difficulty in predictably quantifying RRA and answering question that suggest that tachycardia alters global repolarization.

Two other potential explanations for the "negative" results deserve note. First, is the issue of statistical power. A power analysis was performed prior to conducting the experiments and as a result we expected to be able to maintain a power level of 0.60 or greater using an effect size of 0.56 (ratio of average observed difference to the variance of observed differences – estimated from pilot studies). Only seven were able to be successfully analyzed due to one animal having data that was not interpretable due to the pacing spike falling on end of T-wave during fixed pacing and then again at similar heart intervals during sinusoidal and random tachypacing evaluation. However, the results do not suggest even a trend for a difference in the QT parameters measured during tachypacing. Accordingly, an underpowered study is not considered a likely explanation for the lack of differential repolarization. Finally, the original theory of a protective effect of atrial fibrillation may simply be wrong.

RECOVERY FROM SHORT-TERM IRREGULAR TACHYPACING IS NOT ASSOCIATED WITH ALTERED QT OR QT DYNAMICS

Neither QT, QT adaption curves, QT variability, beat-to-beat QT variability, or T_pT_e are differentially affected between random and sine wave tachypacing. Due to the potential error introduced by heart rate correction, these data were analyzed using the absolute

QT interval. Although the natural heart rates following termination of the three types of tachypacing are similar, small changes in the QT interval may be inadvertently missed. The most effective solution is to introduce fixed-rate pacing at a basal rate lower than that was used for used to comparing sinusoidal and random tachypacing to supraventricular arrhythmia. This would then permit the evaluation of dynamical changes in QT, with a high degree of reliability, so that the main effect would be QT interval modulation. This method was used for subsequent experiments.

The dynamics of repolarization has been assessed in numerous studies that examine beat-to-beat variability of repolarization (BVR) and the QT variability index (QTvi). Poincare plots are used to represent BVR visually and were found in the studies in this dissertation to be highly variable between animals. Thomsen et al. used BVR to predict *d*-sotolol induced RRA in dogs (2004). Similarly, the block of I_{Kr} and I_{Ks}, a known combination that promotes risk for RRAs, was predicted by BVR (Lengyel, 2007). This is also predictive for RRAs in experimental pharmaceutical compounds (e.g. AZD1305), and summarized in an interesting study by Hinterseer et al., who found that the BVR of the QT interval is increased in human patients with drug-induced LQT syndrome (Carlsson et al., 2009; Hinterseer et al., 2008). Ronald Berger at the Johns Hopkins University developed the formula for QT interval variability (QTvi) as the log-ratio between the QT interval and heart rate variability, each normalized by the squared mean of the respective time series, as a measure of RRA risk (Berger et al., 1997). These studies in normal pigs did not result in values that met statistical significance

between sinusoidal and random tachypacing. The fixed pacing group was excluded from these comparisons because, during pacing, the heart rate variation was zero, making the application of the formula impossible. Most application of QTvi has been to subjects in sinus rhythm. Indeed, little is known about its predictive power during real or simulated atrial fibrillation.

SERUM DOFETILIDE CONCENTRATION IS DECREASED IN RANDOM TACHYPACING COMPARED TO FIXED OR SINUSOIDAL

An initial examination of the data indicates a possible mechanism for protection during atrial fibrillation because dofetilide exposure is a proarrhythmic risk factor. During irregular tachypacing, the serum dofetilide concentration was significantly reduced; a finding not observed for either fixed or sinusoidal tachypacing. These studies were not designed to identify a mechanism or explanation for this unexpected result. Rapid onset and reversible effects on blood pH, and thus dofetilide assay accuracy, or tachypacing mediated shifts across fluid compartments are speculative mechanisms that might explain this interesting finding.

Due to the QT prolonging effects of dofetilide, a parallel fall in QT interval would have been predicted during the time of reduced serum concentration in the randomly paced group. This did not occur. However, it is possible that the strong reverse-use dependency of dofetilide attenuates any apparent change in QT interval responsiveness. It is possible that at lower irregular tachycardic rates, this reduction in

serum dofetilide concentration might be associated with changes in QT intervals that could confer some protection. Darbar et al., found that the cycle length to QT interval relationship was flat in humans experiencing atrial fibrillation so the lack of a corresponding change in QT might not indicate the absence of a parallel electrophysiologic effect (Darbar et al., 2004).

While the results of these studies in pigs did not directly support a protective effect of tachycardic irregularity, the changing dofetilide concentrations are interesting and deserve further experimental attention. The altered pharmacokinetics of dofetilide during random tachypacing should be further explored as a possible explanation for the putative clinically observed atrial fibrillation protection. There is a lack of data in the literature on how dofetilide exposures are altered during tachypacing. Enhanced metabolism does not explain the observed findings because the serum concentrations recover to pre-tachypacing levels after the termination of tachypacing.

DOFETILIDE ALTERS REPOLARIZATION, BUT DOES NOT ALTER IT DIFFERENTIALLY FOR RANDOM VERSUS SINUSOIDAL TACHYPACING

The findings did not support an effect of dofetilide to alter repolarization in the post-irregular pacing period in a way that would suggest increased risk for RRAs relative to sinusoidal tachypacing. Despite this, during the post-tachycardia period, both sinusoidal and random pacing have lengthened QT intervals compared to control pigs

that were paced at 150 beats/min. This data suggests that tachycardia, and not irregularity per se, confers heightened risk for RRAs or proarrhythmia.

This post-tachypacing return to prolonged QT intervals has been reported for patients with atrial fibrillation converted to sinus rhythm. The mechanism appears to be more complex than simply a return to previously established QT duration as heart rate falls, since the QT interval of the dofetilide treated control group steadily declines over time. This is not seen in the tachypacing groups which experience a sustained QT prolongation, even one hour after cessation of the induced tachycardia. Differential dofetilide exposure does not explain this disparity since all groups had similar (and slightly declining) serum concentrations over time. Whatever the mechanism involved, the results of these studies indicate that protection and post-conversion vulnerability could be a result of rate related alterations.

The post-tachypacing QT finding has to be interpreted in light of the methodologies employed. Because of the limitations for heart rate correction formulas mentioned earlier, the post-tachypacing analyses had to be conducted at a selected, fixed heart rate. The selection of a pacing rate for this analysis could induce some bias. A rate of 150 beats/min might be non-ideal for detecting post conversion risk. This rate issue is also likely to be somewhat species specific. Typical resting heart rates for humans are about 80 beats/min, a rate that is 40 beats/min lower than the average human ventricular rate during atrial fibrillation (120 beats/min). In pigs the resting heart rate is higher than humans (116$\pm$4.3 beats/min). Therefore, pigs have a natural heart rate that

is roughly one-third to one-half greater than adult humans. The average heart rate for tachypacing in the final arm of these studies was 180 beats/min. This increase is 1/3 higher than the average ventricular rate for humans with untreated atrial fibrillation. This indicates that the findings within this dissertation are likely relevant to the human disease.

Atrial fibrillation has a *natural* heart rate that is higher than the heart rate during sinus rhythm. A well established risk factor for RRA is a slow heart rate. Therefore, it is logical that tachycardia itself may provide protection from the development of arrhythmia. Indeed, it has been shown that temporary pacing, at rates of 90-100 beats/min, can acutely prevent RRAs (Sclarovsky et al., 1979; DiSegni et al., 1980). However, the mechanism for this has not been defined The QT interval lengthening in pigs observed after the termination of tachypacing (both sinusoidal and random), persists for the duration of the monitoring period (one hour). Because of a lack of preliminary evidence, experiments were not designed to monitor for QT interval prolongation for over one hour. Although the QT interval prolongation findings are statistically significant, the physiologic relevance needs to be considered. Bauman et al., noted in a 23-patient study that 74% of RRAs occurred within one week from the conversion from atrial fibrillation to sinus rhythm (Bauman et al., 1984). In an 11 patient study, Roden et al., demonstrated that 75% of RRAs occurred within three days or less (Roden et al., 1986). However, other reports indicated that the average time to RRA may be 4.7 years (Oberg et al., 1994). This conflicting evidence suggests that alterations in repolarization lasting only seconds to minutes may not be physiologically

meaningful.

It may be that fast heart rates alone may be responsible for the presumed protection during atrial fibrillation because the conversion of atrial fibrillation is often (but not always) accompanied by a sudden slowing in ventricular rate. In a study, involving 12 subjects, the extent of QT interval prolongation was examined during intravenous dofetilide administration. It was found that, despite there being no heart rate effect, the extent of QT prolongation in the dofetilide treated group, was greater than in the group converted to sinus rhythm (Choy et al., 1999).

The results for excess QT interval prolongation observed in the studies performed at Vanderbilt may represent a unique population (the patients with both atrial fibrillation and sinus rhythm had equal heart rates) (Choy et al., 1999; Darbar et al., 2004; Darbar et al., 2008). It is unusual to have ventricular rates that are equivalent during sinus rhythm and atrial fibrillation. During the conversion of atrial fibrillation to sinus rhythm, it is far more common to observe a drop in heart rate. Because of the rate dependence of the QT interval, it can be predicted that a corresponding increase in QT duration will be observed after conversion to sinus rhythm. In the Vanderbilt studies, it was noted that patients had a marked increase in QT interval duration after conversion to sinus rhythm, despite an attenuated heart rate change (Choy et al., 1999). These findings may be explained by an epiphenomenon that is predicated by a subclinical mutation that decreases repolarization reserve. In our studies, sinusoidal and randomly paced pigs

treated with dofetilide exhibited a decreased QT interval trend that was almost identical to the dofetilide treated control pigs paced at 150 beats/min. Preliminary predictions were that tachypacing would produce further QT interval reductions compared to control pigs. However, this attenuated QT interval reduction is supported by findings in humans that dofetilide attenuates the QT interval increase during atrial fibrillation (Darbar et al., 2004). Following the termination of sinusoidal and random tachypacing in dofetilide treated pigs, the QT interval significantly increased. The increased QT interval occurs in patients following dofetilide-induced conversion of atrial fibrillation to sinus rhythm (Choy et al., 1999). The changes we observed did not suggest that there was a difference between short-term sinusoidal or random tachypacing following its termination. Yet these findings are provocative because, compared to pigs treated with dofetilide and paced at 150 beats/min, both of these tachypacing treatments significantly increased. This directly supports the theory that tachypacing alone (with dofetilide treatment) may alter repolarization favoring risk following the conversion of atrial fibrillation to sinus rhythm. It remains possible that the random variation control (i.e. sinusoidal pacing) may provide the substrate to the heart, equivalent to random pacing. Perhaps the hearts physiology is altered due to a difference in cycle length that is not differentiated by the application of this particular type of sinusoidal tachypacing.

Repolarization itself has been shown to be a dynamic feature, exemplified by a study in by Xiao et al. showing that paced ventricular myocytes treated with dofetilide have shorter action potential durations and a depressed dofetilide response (Xiao et al., 2008). One controversial theory has been that antagonism of I_{Kr} may lead to

compensatory up-regulation of I_{Ks} (Lou et al., 2007). This suggests that repolarization may be regulated through feedback ion channel functioning, and altered by disease or chronic drug treatment. These factors should be taken into account by pharmacologists involved in drug discovery and toxicology.

CHAPTER SEVEN - SUMMARY

Data presented in this dissertation support results from clinical studies that suggest heart rate irregularity may contribute to a protection or post-conversion vulnerability. In this research, an atrial tachypacing model capable of mimicking the irregularity of atrial fibrillation was established. This irregularity is a defining and unique characteristic of atrial fibrillation. Once this model was refined, it was used to assess whether irregular atrial tachypacing alters repolarization dynamics differently that repetitive sinusoidal tachypacing. This repetitive sinusoidal tachypacing had a variance and average heart rate equivalent to irregular tachypacing. Sinusoidal, irregular, and fixed-rate atrial tachypacing treatments were administered to *normal*, anesthetized domestic pigs, and demonstrated that irregular short-term tachypacing fails to differentially alter commonly used repolarization parameters, either during or after the termination of tachypacing. Having established this finding, dofetilide was selected to block the I_{Kr} current, which has proven to be involved in nearly all forms of drug-induced proarrhythmic liability. The hypothesis tested was that dofetilide would unmask effects of irregular tachypacing that are not evident in untreated normal animals. Due to the lack of available data in the literature, the pharmacokinetics and pharmacodynamics of dofetilide was evaluated in pigs. The data was used to establish an intravenous dofetilide administration protocol that produced steady-state serum concentrations, which allowed the study of the effect of dofetilide on short-term atrial tachypacing in pigs.

The main findings described in this dissertation are summarized as follows.

During tachypacing, neither QT interval nor QTvi were significantly different between tachypacing groups. Similarly, in the post-tachypacing period, none of the repolarization parameters tested was differentially affected by the type of tachypacing. These results do not support an effect of irregular (or any type) tachypacing on repolarization that would likely confer protection against RRAs.

Dofetilide, when administered to pigs, is eliminated in a dual kinetic process in a pattern that has not been reported before.

Dofetilide, an antiarrhythmic that is also proarrhythmic, has a lower serum concentration during random tachypacing compared to sinusoidal tachypacing. Though this suggests a possible mechanism for a protective effect during irregular tachycardias like atrial fibrillation, the reduction in dofetilide concentrations were not accompanied by evident changes in repolarization. The significance of this finding requires additional investigation.

Random and sinusoidal tachypacing in dofetilide treated pigs is associated with an increase in the RT interval just after the termination of tachypacing that persists for at least 60 minutes. This persistent elevation in QT interval is different for both forms of tachypacing relative to control animals. This suggests that tachycardia of any type may confer a post-tachycardia vulnerability to RRAs due to a fast, rather than an irregular,

heart rate. This post tachypacing QT prolongation was only observed in the presence of I_{Kr} blockade, suggesting an important combined effect of tachycardia and drug induced I_{Kr} inhibition.

FUTURE STUDIES

The experiments described in this dissertation use repolarization parameters as a surrogate for risk for RRAs. Future experiments could include a direct evaluation for the role of irregularity in the development of RRAs in animals treated with dofetilide. The selection of an animal model seems to be important for experiments involving dofetilide, as supported by the pharmacokinetic data in this dissertation that suggests a novel dual-effect elimination system. This line of study would focus on understanding whether QT interval changes or altered dofetilide exposure, change the risk for RRAs. Such experiments require an animal model with predictable ability to generate RRAs, even in the context of drug (dofetilide) and atrial fibrillation or random tachypacing. If the prediction of a lower risk for RRA during random tachypacing is validated, then mechanistic studies would involve altering the dofetilide serum concentration during random pacing so that it is equivalent to control or sinusoidal pacing. The hypothesis for this future study would be that the risk for RRA would be equal to control and sinusoidal pacing. If the QT interval attenuation is then restored during heightened dofetilide exposure, then it suggested that the risk alteration is mediated though heightened exposure to dofetilide and that the dofetilide exposure is strongly coupled to the repolarization currents responsible for QT interval prolongation.

Understanding more fully the role of fixed-rate tachypacing during dofetilide treatment is another important future experiment. Fixed-rate tachypacing at rates equal to the average for sinusoidal and random would be administered for one hour. If the post-tachypacing QT interval prolongation is not observed, it indicates that alterations in heart rate are important to post-tachycardia QT interval lengthening. Additional studies would be performed and aimed at understanding the precise rate alteration that induces a QT interval prolongation.

The details relating to I_{Kr} block, action potential prolongation, QT interval lengthening, and RRA risk, are a superb model for translational science. The work toward understanding the role for random rate tachycardias elucidated here is an example of findings that may add some clarity in this field. Proarrhythmia has formed the vulnerable point in drug treatment for atrial fibrillation and other arrhythmias. Based on findings from this dissertation and expert research in this field, it is hoped that drug compounds can be found that reduce the potential for RRAs.

APPENDICES

APPENDIX A - COMPARISON OF HEART RATE – QT INTERVAL RELATIONSHIPS BETWEEN CONSCIOUS AND ISOFLURANE ANESTHETIZED NORMAL DOMESTIC PIGS

INTRODUCTION

Numerous recent publications have pointed to the inadequacy of using a single heart rate correction formula for all species and under all conditions (Malik, 2001; Malik, 2002; Desai et al., 2003; Shah & Hajian, 2003; King et al., 2006). Studies involving the measurement of the QT interval often rely on having a robust heart rate correction formula that normalizes the QT interval to the heart rate (Soloviev et al., 2006). This enables a convenient surrogate measure of repolarization independent of heart rate-related repolarization alterations. Although imperfect, the corrected QT interval acts as a surrogate for proarrhythmic risk (Haverkamp et al., 2000).

Studying the QT interval in pigs can play an important role in the assessment of compounds or other interventions that have effects on cardiac repolarization. Due to the similarity to humans in cardiac anatomy and ion channel distribution, domestic pigs are an acceptable model for conduction-related studies (Bharati et al., 1991). This is in part due to their surface area to organ ratio and electrophysiology being similar to those of humans (Rubart et al., 1997; Kates et al., 1984), and in part due to the similarity of the coronary vessels' reactivity and adrenergic receptor distribution between pigs and humans, and dissimilarity to other model systems (Hidaka et al., 1985). Previous studies suggest that pigs can be used as a suitable model of human disease for the study of drug-induced QT interval prolongation (Kano et al., 2005).

The increased proarrhythmic risk associated with prolongation of the QT interval is an important parameter that is monitored by the US Food and Drug Administration (FDA) for new drug compounds. Indeed, a QT increase of 6 msec is used by the FDA as a threshold for heightened concern for repolarization-related arrhythmia or Torsades (E14 Clinical Eval QT/QTc, 2005). Previous work has demonstrated that the degree of correction formula-induced error similar to, or greater than the 6 msec concern threshold. Therefore, the error introduced from a correction formula that is non-optimal may confound the interpretation of proarrhythmic risk (King et al., 2006).

While awake, pigs can be difficult to manipulate; therefore experiments are often conducted while the subjects are anesthetized. Isoflurane is a commonly used anesthetic used for the induction and maintenance of general anesthesia. Isoflurane is known to reduce contractility, depress cardiac output and alter autonomic tone, although it is one of the least cardiotoxic anesthetics (Gross, 2009). In dogs, every volatile anesthetic induces QT prolongation and isoflurane causes QTc prolongation independent of the animal's autonomic state (Riley, 1988).

Little is known about how cardiac repolarization in the pig is modified by isoflurane-based anesthesia. The objective of this study is to evaluate the QT interval as a repolarization parameter, and to establish the heart rate / QT interval relationship, during caged, unanesthetized periods and compare that to a time period during general anesthesia. We hypothesize that pigs under isoflurane anesthesia with have a different

heart rate / QT relationship than while caged, and the optimum correction formula while caged will be different than during isoflurane anesthesia.

METHODS

ANIMALS

This study was approved by the All University Animal Care and Use Committee of Michigan State University and conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

Farm raised female pigs were acquired from facilities located at Michigan State University. Food and water was supplied ad libitum. Animals were studied prior to reaching sexual maturity and had similar weights (average body weight: 34 $\pm$ 4 kg). All animals were acclimated prior to data collection for at least 48 hours.

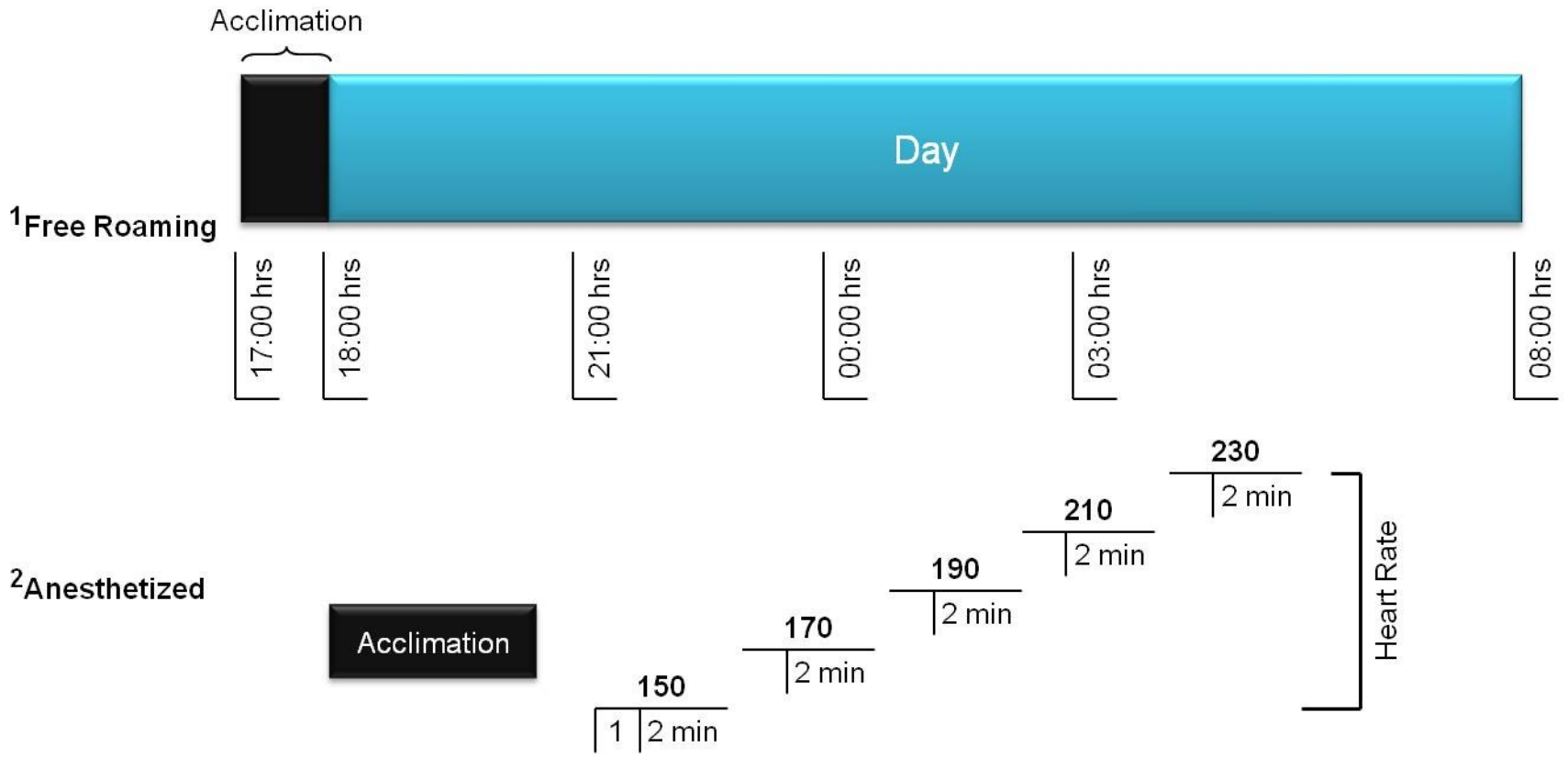
DATA COLLECTION

Caged Experiments

Unanesthetized studies were performed with the animal in isolation from human contact, fitted with jackets, surface electrocardiogram (ECG) electrodes, and radiotelemeters. Data collection was for 14 hours and consisted of an equal light and dark cycle in order to balance potential circadian mediated QT effects and to minimize

the exposure to noise from caretakers. Data collection began at 6PM and continued until 8AM the following day (Figure A.1).

Figure A.1 Study Design including the first phase for pigs that are caged[1] (and unanesthetized (Free Roaming) and second phase conducted in pigs anesthetized with isoflurane (Anesthetized)[2].



Data collection was performed gathering ECG data in a modified lead configuration pattern for analysis of repolarization and heart rate parameters. The Nehb-Spori lead system (position 1) was used for analysis because it has been shown to allow the greatest precision for marking the T-end in the caged-unanesthetized pig (Nahas et al., 2002). The Nehb-Spori lead corresponding to standard lead II was used for the heart rate / QT assessment.

Anesthetized Experiments

Anesthesia was induced and maintained by inhaled isoflurane (Abbott Laboratories, Chicago, IL) mixed with oxygen. Following endotracheal intubation the animal was placed on a ventilator (Hallowell EMC, Pittsfield, MA) at a rate (18 $\pm$ 2 breaths/minute) and tidal volume (400 $\pm$ 75 ml) to maintain an end tidal CO_2 (Datascope Corp, Paramus, NJ) of 35-45 Torr. Intravenous access was achieved through the ear vein and external jugular vein. Intravenous isotonic fluid (Lactated Ringers) was administered at a rate of 2.2 ml/kg/hour.

Pacing was performed with a dacron electrophysiology catheter (St. Jude Medical, Minneapolis, MN) advanced into the high right atria through a jugular vein that was isolated and accessed surgically. The position was confirmed with fluoroscopy and the voltage for stimulation (SD9 - Grass Product Group, W. Warwick, RI) was established based on doubling the pacing threshold. ECG signals were collected at a sample rate of 1 kHz on a digital recording system using lead V1 for heart rate / QT interval analysis. Subjects underwent a high right-atrial tachypacing ramp at 150, 170, 190, 210 and 230

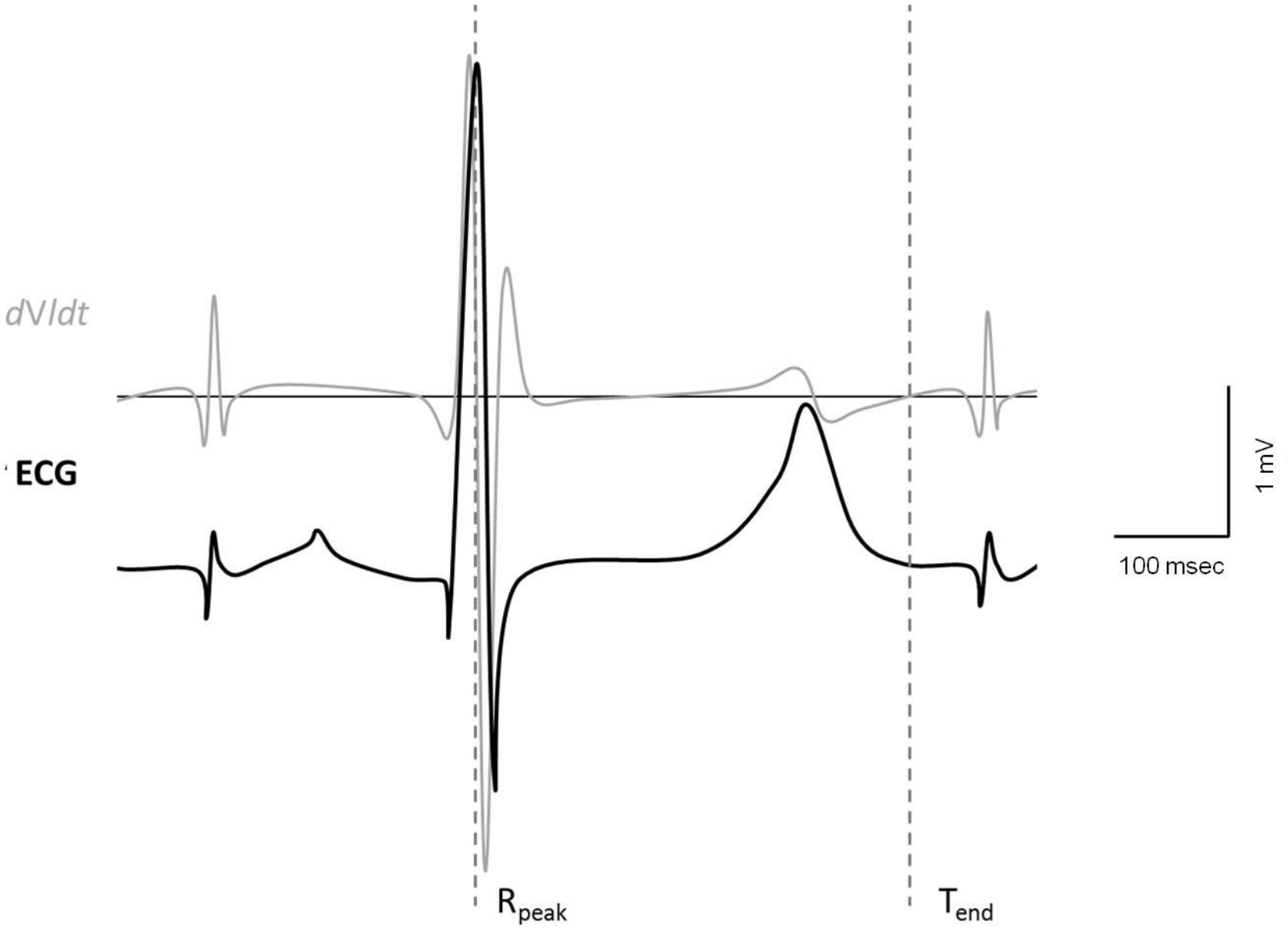
beats per minute for three minute durations. The first minute was excluded from the analysis due to the potential for bias stemming from any repolarization memory effect (Figure A.1). Baseline parameters were analyzed following one hour of isoflurane anesthesia using the exact Nehb-Spori lead configuration and telemetry system used for the caged data collection.

ANALYSIS

Data was acquired using EMKA-Iox (v1.8.9.9) and interval measurements performed on EMKA-ECG auto version 2.5.1.31 (EMKA Technologies, Paris, France). EMKA utilizes a shape-based algorithm to identify the beginning and ending of time periods. Template beats were extracted to form a library consisting of the minimal number of beats (3 ± 2) to reach an acceptable yield and for the data for heart rate step changes, a model beat was used for each of the five step changes. All interpretable ECG complexes were taken over 14 hours for the free roaming and the final 2 of 3 minutes at each paced, heart rate step increment. Baseline data was collected during anesthetized during a 15 minute period prior a pacing intervention. We considered the successful evaluation of a minimum of 80% of recorded intervals to be an acceptable yield. The RT interval was substituted for the QT interval to improve the imprecision based on marking the beginning of the Q-wave fiduciary point. The R-wave, T-peak and the end-of-T were identified by a zero-slope, first derivative function derived from the ECG (Figure A.2).

Figure A.2 Representative fiduciary points from the automated morphology-based analysis of the lead V1 electrocardiogram (dark tracing) comparing the RT interval with the corresponding *d*V/*dt.* The lighter tracing represents the first derivative of the original electrocardiogram with corresponding isoelectric line. The dotted lines are the fiduciary points used to mark the beginning and end of the RT interval.

Figure A.2 (Cont'd)



The central tendency of the data is presented as mean plus or minus the standard error of the mean (SEM). A paired t-test was used to compare baseline electrocardiographic data between caged and isoflurane anesthesia conditions and the residual slopes between treatments. Differences were considered to be significant at $p<0.05$.

RESULTS

BASELINE CHARACTERISTICS

Electrocardiographic parameters relevant to repolarization are summarized in Table A.1. The QT and RT interval was significantly different between caged pigs and pigs under isoflurane-based anesthesia, while the remaining parameters (heart rate and T_{peak} to T_{end}) were not significantly different.

Table A.1 Summarized values for measured electrocardiographic parameters, instantaneous heart rate (beats per minute), R to R interval (msec), QT/RT interval (msec), the peak of T to the end of T wave (T_{peak} – T_{end}), and the duration of the QRS complex in caged and isoflurane anesthetized domestic pigs (n=9).

Baseline ECG Parameters					
	Caged	**SEM**	**Anesthetized**	**SEM**	**P-value**
Heart Rate	119.6	0.15	106.9	0.09	0.19
R-R Interval	515.9	0.63	576.1	0.43	0.15
QT Interval	270.3	0.13	309.3	0.10	0.04*
RT Interval	254.1	0.13	289.9	0.10	0.02*
T_{peak} - T_{end}	42.5	0.03	46.6	0.04	0.92
QRS Duration	52.6	0.03	57.1	0.02	0.15
PR Interval	100.0	0.06	97.6	0.05	0.37

CAGED HEART RATE – RT AND RTC INTERVAL RELATIONSHIP

The slope of the heart rate / RT relationship within the data gathered under caged unanesthetized conditions compared to the same data limiting the heart rate to 150 to 230 beats/min was not significantly different (-0.76±0.08 versus -1.32±0.11, respectively). Following the application of formula based correction, various formulas over and under-corrected for heart rate based on the residual slope. Table A.2 shows the residual slope comparison between full and limited heart rate conditions for caged animals with each of 29 correction formulas applied. The variance between subjects for each of these correction formulas is high between the full and limited heart rate data (Figure A.4).

Figure A.3 Representative heart rate – corrected RT interval graph reflecting an example of over and under correction for heart rate related RT interval changes following the application of 28 commonly used correction formulas. Key: Sarma (a); Boudolas (b); Kovacs (c); Rickards (d); Mayeda (e); Arrowood (f); Lecocq2 (g); Sarma/Hodges (h); Lecocq1 (i); Rautaharju1 (j); Rautaharju2 (k); Klingfield (l); Bazett (m); Wohlfart (n); Yoshinaga (o); Schlamowitz (p); Ljung (q); Boudolas-logarithmic (r); Hodges exponential (s); Adams (t); Framingham (u); Simonson (v); Friericia (w); Simonson logarithmic (x); Larsen & Skulason (y); Todt (z); Kawataki (aa); Van de Waters (bb). [The text in this figure is not meant to be decipherable, but for visual reference only.]

Figure A.3 (Cont'd)

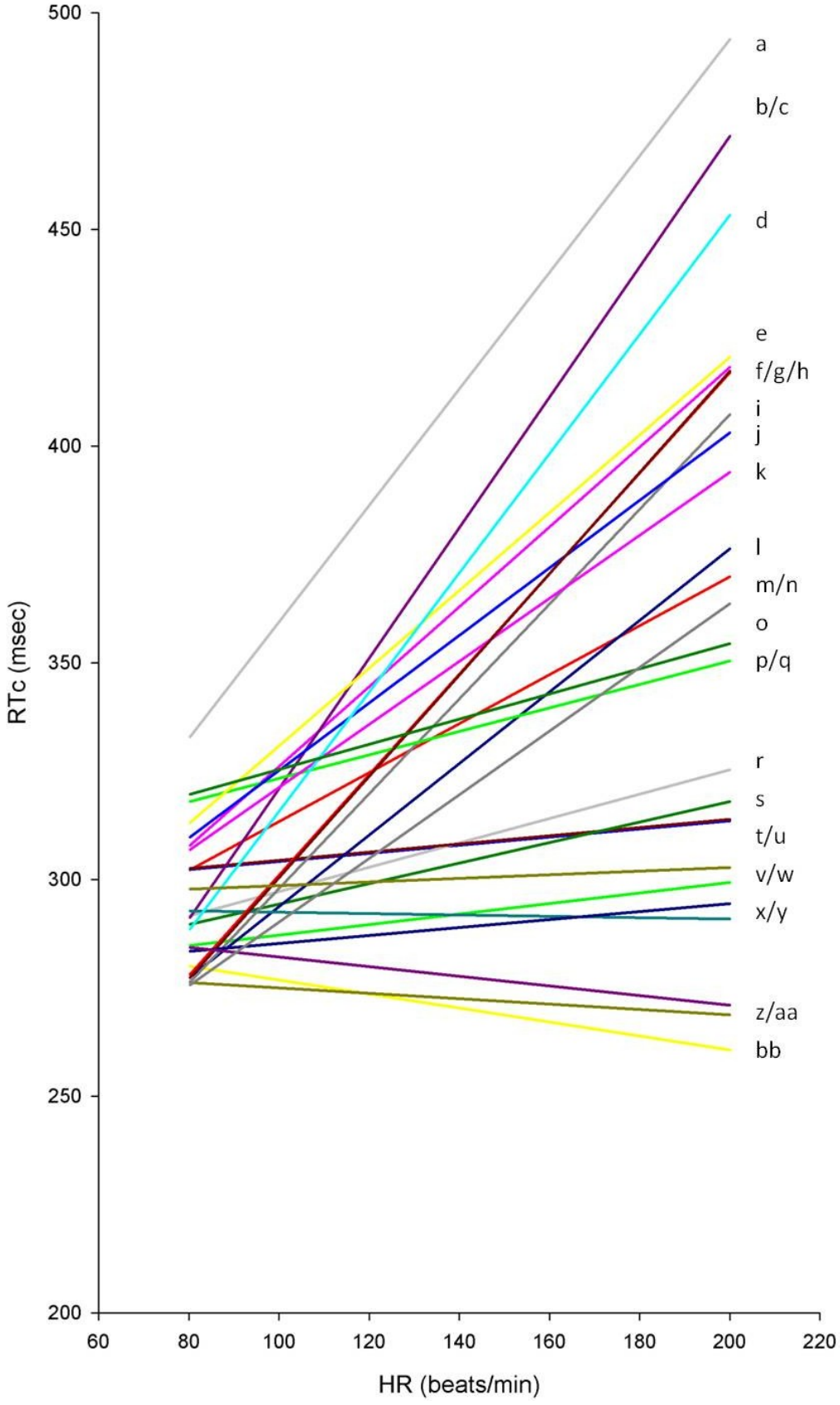


Table A.2 Twenty-nine commonly used QT interval correction formulas and the application of these formulas, using the RT interval, to the linear regression of the heart rate-RT interval data in caged pigs (n=10). RR is the time interval between successive QRS complexes in seconds; HR is the instantaneous heart rate in beats per minute; QT is the interval from the beginning of the QRS complex to the end of the T wave in seconds. Adams, 1936(1); Arrowood et al., 1993(2); Bazett, 1920(2); Boudoulas, 1981(4); Fridericia, 1920(5); Sagie et al., 1992(6); Hodges, 1997(7); Hodges et al., 1983(8); Kawataki et al., 1984(9); Klingfield et al., 1995(10); Kovacs, 1985(11); Larsen & Skulason, 1941(12); Lecocq et al., 1989(13); Lijung, 1950(14); Mayeda, 1934(15); Rautaharju et al., 1990(16); Rickards & Norman, 1981(17); Sarma et al., 1984(18); Schlamowitz, 1946(19); Simonson et al., 1962(20); Todt et al., 1992(21); Van de Water et al., 1989(22); Wohlfart & Pahlm, 1994(23); Yoshinaga et al., 1993(24).

Correction Formula	Equation (QT_C=)	Entire Heart Rate Residual Slope (±SEM)	Limited Heart Rate Residual Slope (±SEM)
Adams[1]	QT + 0.1536 (1-RR)	0.1509 (± 0.0513)	0.3797 (± 0.0649)
Arrowood[2]	QT + 0.304 – 0.492$e^{-0.008\text{^}HR}$	0.7270 (± 0.0513)	0.3797 (± 0.0649)
Bazett[3]	QT / $RR^{0.5}$	0.4387 (± 0.0819)	0.2073 (± 0.0760)
Boudolas[3]	QT + (2 / 1000)(HR – 60)	1.2216 (± 0.1144)	1.0943 (± 0.1419)
Boudolas-logarithmic[4]	QT / $RR^{0.398}$	0.2273 (± 0.0651)	0.3011 (± 0.0821)
Fridericia[5]	QT / $RR^{0.333}$	0.1859 (± 0.0592)	0.3861 (± 0.0787)
Framingham[6]	QT + 0.154 (1 – RR)	0.1507 (± 0.0514)	0.3790 (± 0.0649)
Hodges exponential[7]	QT / $RR^{0.38}$	0.2089 (± 0.0617)	0.3259 (± 0.0810)

Table A.2 (Cont'd)

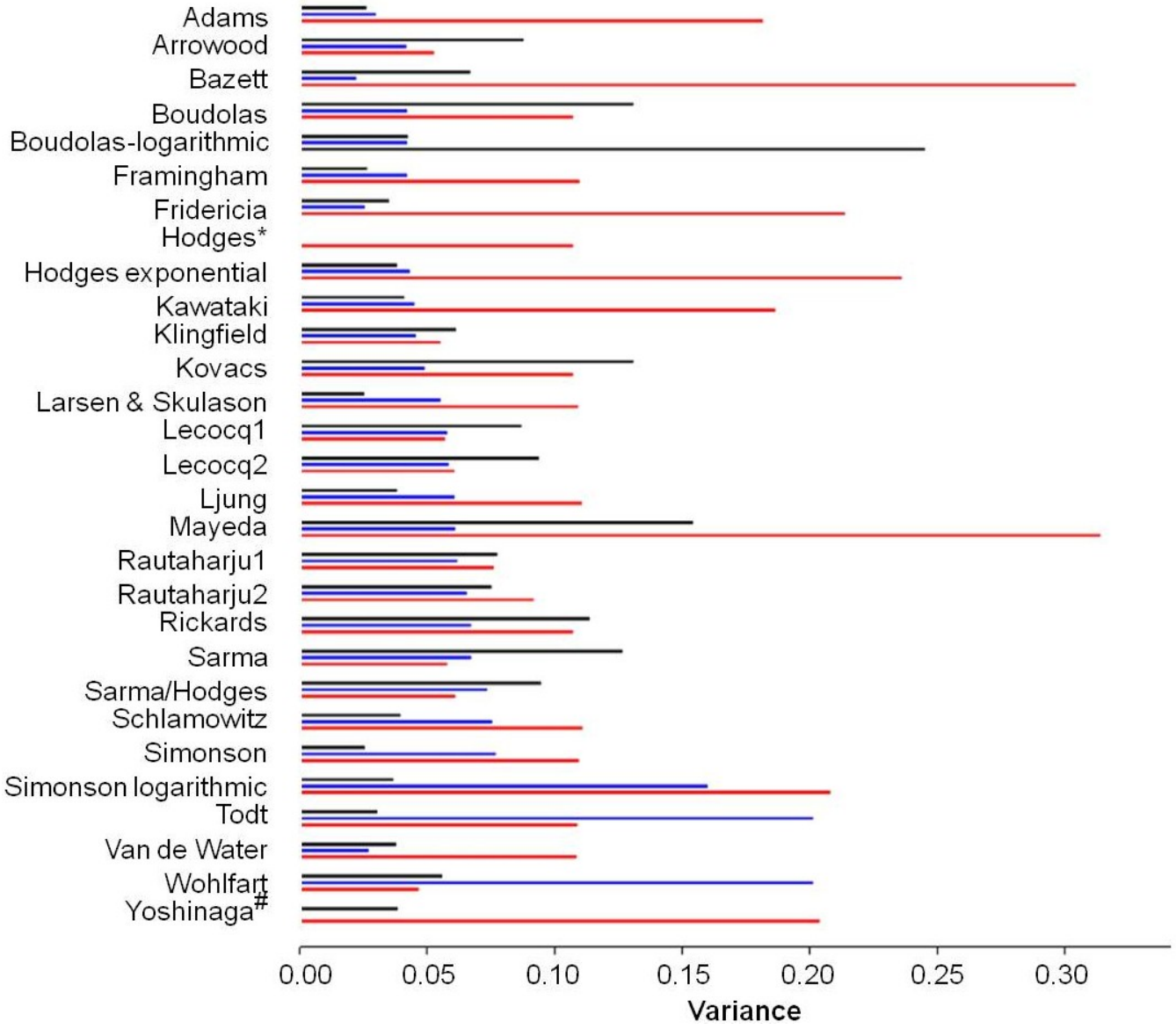
Hodges[8]	QT + (1.75 / 1000) (HR – 60)	6.6799 (± 1.0534)	3.3918 (± 0.5825)
Kawataki[9]	$Qt / RR^{0.25}$	0.2542 (± 0.0640)	0.4775 (± 0.0764)
Klingfield[10]	Qt + (1.32 / 1000) (HR – 60)	0.5915 (± 0.0783)	0.5544 (± 0.0743)
Kovacs[11]	QT – 0.12 + (0.12 / RR)	1.2217 (± 0.1144)	1.0943 (± 0.1419)
Larsen & Skulason[12]	QT + 0.125 (1 – RR)	0.2003 (± 0.0505)	0.4316 (± 0.0650)
Lecocq1[13]	$QT – 0.017 + 0.67e^{-3.7\text{^}RR}$	0.8398 (± 0.0933)	0.6497 (± 0.0820)
Lecocq2[13]	$QT – 0.017 + 0.704e^{-3.7\text{^}RR}$	0.9008 (± 0.0968)	0.6971 (± 0.0870)
Ljung[14]	QT + 0.2 (1 – RR)	0.2101 (± 0.0618)	0.2955 (± 0.0671)
Mayeda[15]	$QT / RR^{0.604}$	0.7064 (± 0.1242)	0.2874 (± 0.0520)
Rautaharju1[16]	QT – (0.656 / 1 – (0.01*HR)) +0.41	0.5993 (± 0.0881)	0.2881 (± 0.0471)
Rautaharju2[16]	$QT + 0.2425 – 0.434e^{-0.0097\text{^}HR}$	0.5507 (± 0.0868)	0.2478 (± 0.0506)
Rickards[17]	QT + (1.87 / 1000)(HR – 60)	1.1012 (± 0.1066)	0.9910 (± 0.1665)
Sarma[18]	$QT – 0.0149 +0.664e^{-2.7\text{^}RR}$	1.1052 (± 0.1125)	0.6914 (± 0.0858)
Sarm/Hodges[8]	$QT – 0.018 + 0.708e^{-3.7\text{^}RR}$	0.9095 (± 0.0973)	0.7038 (± 0.0878)

Table A.2 (Cont'd)

Schlamowitz[19]	QT + 0.205 (1 - RR)	0.2249 (± 0.0630)	0.2864 (± 0.0675)
Simonson[20]	QT + 0.14 (1 – RR)	0.1668 (± 0.0507)	0.4044 (± 0.0648)
Simonson logarithmic[20]	$QT / RR^{0.32}$	0.1849 (± 0.0607)	0.4017 (± 0.0782)
Todt[21]	QT + 0.1 (1 - RR)	0.2699 (± 0.0551)	0.4770 (± 0.0659)
Van de Water[22]	QT + 0.087 (1 – RR)	0.3061 (± 0.0616)	0.4956 (± 0.0672)
Wohlfart[23]	QT + (1.23 / 1000)(HR – 60)	0.5080 (± 0.0747)	0.4829 (± 0.0700)
Yoshinaga[24]	$QT / RR^{0.31}$	0.1868 (± 0.0619)	0.4133 (± 0.0778)

Figure A.4 Observed variance of the slope between subjects (n=10) following the application of correction formulas from data in caged natural hear rate (black), heart rate limited conscious (blue), and heart rate limited anesthetized (red) pigs. Variance values for Hodges (*1109.6 and 339.3, respectively) and Yoshinaga (# 20.1) are not shown due to being non-characteristically high. [The text in this figure is not meant to be decipherable, but for visual reference only.]

Figure A.4 (Cont'd)



ANESTHETIZED HEART RATE – QT AND QTC INTERVAL RELATIONSHIP

Following the application of formula based correction, various formulas over and under-corrected for heart rate based on the residual slope. Table A.3 shows the residual slope, with each of 29 correction formulas applied, for correction formulas applied to pigs undergoing pacing-induced step changes in heart rate. The variance of the slope between subjects for each of these correction formulas is highly variable between caged and anesthetized with a limited heart rate data (Figure A.4).

Table A.3 Twenty-nine commonly used QT interval correction formulas applied, using the RT interval, to the uncorrected heart rate – RT interval data and the residual slope following correction in anesthetized pigs (n=10).

Correction Formula	Anesthetized Residual Slope (± SEM)	Correction Formula	Anesthetized Residual Slope (± SEM)
Adams[1]	0.8943 (±0.1348)	**Ljung**[14]	0.6828 (± 0.1052)
Arrowood[2]	0.2801 (±0.0727)	**Mayeda**[15]	0.6436 (± 0.1772)
Bazett[3]	0.7500 (±0.1745)	**Rautaharju1**[16]	0.2956 (± 0.0873)
Boudolas[3]	0.9680 (± 0.1035)	**Rautaharju2**[16]	0.3719 (± 0.0959)
Boudolas-logarithmic[4]	0.8613 (± 0.1566)	**Rickards**[17]	0.8380 (± 0.1035)
Fridericia[5]	0.9147 (±0.1462)	**Sarma**[18]	0.3449 (± 0.0761)
Framingham[6]	0.7631 (± 0.1048)	**Sarma / Hodges**[8]	0.3950 (± 0.0781)
Hodges exponential[7]	0.8773 (± 0.1536)	**Schlamowitz**[19]	0.6741 (± 0.1053)
Hodges[8]	0.7180 (± 0.1035)	**Simonson**[20]	0.7876 (± 0.1047)
Kawataki[9]	0.9346 (± 0.1366)	**Simonson logarithmic**[20]	0.9239 (± 0.1442)
Klingfield[10]	0.3599 (± 0.0744)	**Todt**[21]	0.8574 (± 0.1043)
Kovacs[11]	0.9675 (± 0.1035)	**Van de Water**[22]	0.8801 (± 0.1042)
Larsen & Skulason[12]	0.8138 (± 0.1045)	**Wohlfart**[23]	0.3058 (± 0.0684)
Lecocq1[13]	0.3498 (± 0.0756)	**Yoshinaga**[24]	0.9307 (± 0.1427)
Lecocq2[13]	0.3889 (± 0.0779)		

DISCUSSION

The inadequacies of QT interval correction have been previously described. The problem extends to domestic pigs as well. A superior, or even consistently better, set of correction formulas that could be used across all experimental conditions was not identified from the data of this study. The pigs of this study were domestic pigs which are only suitable for short term studies, because of their rapid growth rates. For chronic research studies, minipigs are more commonly used. Further work would be needed to determine if the results of this study are applicable to other porcine strains. This study supports the state dependence of the heart rate – QT interval relationship. The formula that corrects for the confounding effects of heart rate changes is different during isoflurane-based general anesthesia than during caged, unanesthetized periods. Isoflurane is a commonly employed drug during experimental procedures, and investigators studying repolarization should be aware of the potential bias when making QT comparisons between these two physiologic states.

Awake and unrestrained pigs, have a high degree of variance in the residual slope of the corrected QT relationship. This was true for all of the correction formulas studied. No single formula in this study was found to be uniquely superior for correction. This is consistent with the Nahas et al., who reported that, individual corrections for Gottingen minipigs were superior to population based correction formulas (Nahas et al., 2002).

Several population based correction formulas performed well by having a residual-slope approximating zero. Specifically, for domestic swine that are not anesthetized, Adams, Framingham and Simonson formulas are preferred. Isoflurane is a commonly used anesthetic that is known to cause prolongation of the QT interval; however, its effect on the heart rate-QT interval relationship has not been resolved. Under this anesthesia, the heart rate / QT interval relationship is altered and different correction formulas provide more robust correction. The top formulas in this study were Arrowood, Rautaharju1 and Wohlfart. However, this relationship was evaluated only for tachycardic heart rates and this interpretation must take that into consideration.

LIMITATIONS

Our pacing ramps were performed under a limited range of tachycardic heart rates. This was done as to allow for overdrive high right atrial pacing. Indeed, while most correction formulas were derived from naturally occurring changes in the QT interval, other possible mechanisms may participate in the heart rate – QT relationship. Care should be used when drawing conclusions from this data outside of these heart rates and in light of the possible bias in accuracy. However, our results are consistent with previous studies that predict that the heart rate – QT relationship would be different depending on the physiologic state (awake versus anesthetized).

APPENDIX B - FIGURE 3.6

Poincare plots as a visual representation of the QT interval beat-to-beat variability of repolarization (BVR) each representing the RT interval as a function of the previous RT interval in a single subject (n=7).

Figure B.1 - Figure 3.6 Graph A Enlarged

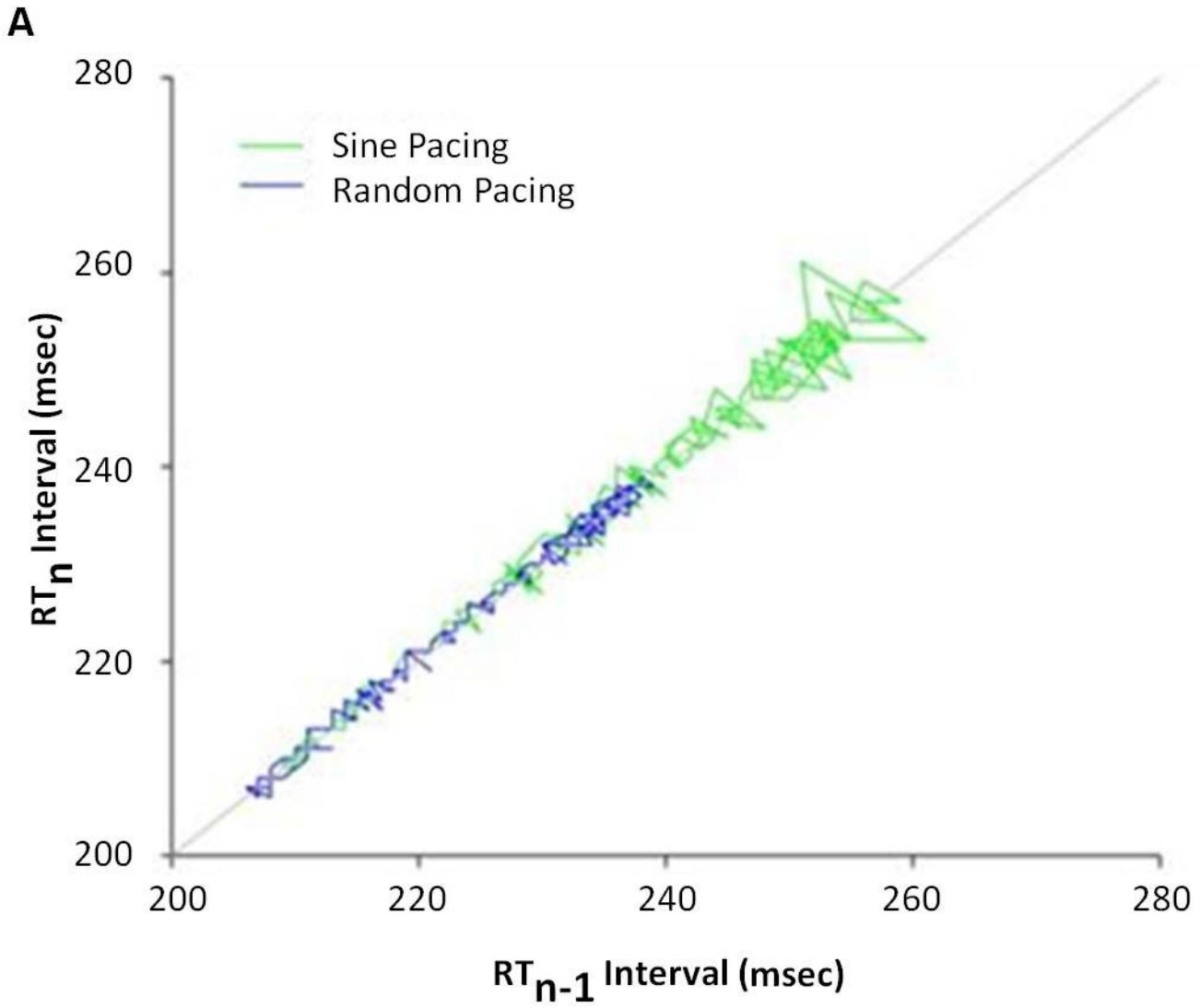


Figure B.2 - Figure 3.6 Graph B Enlarged

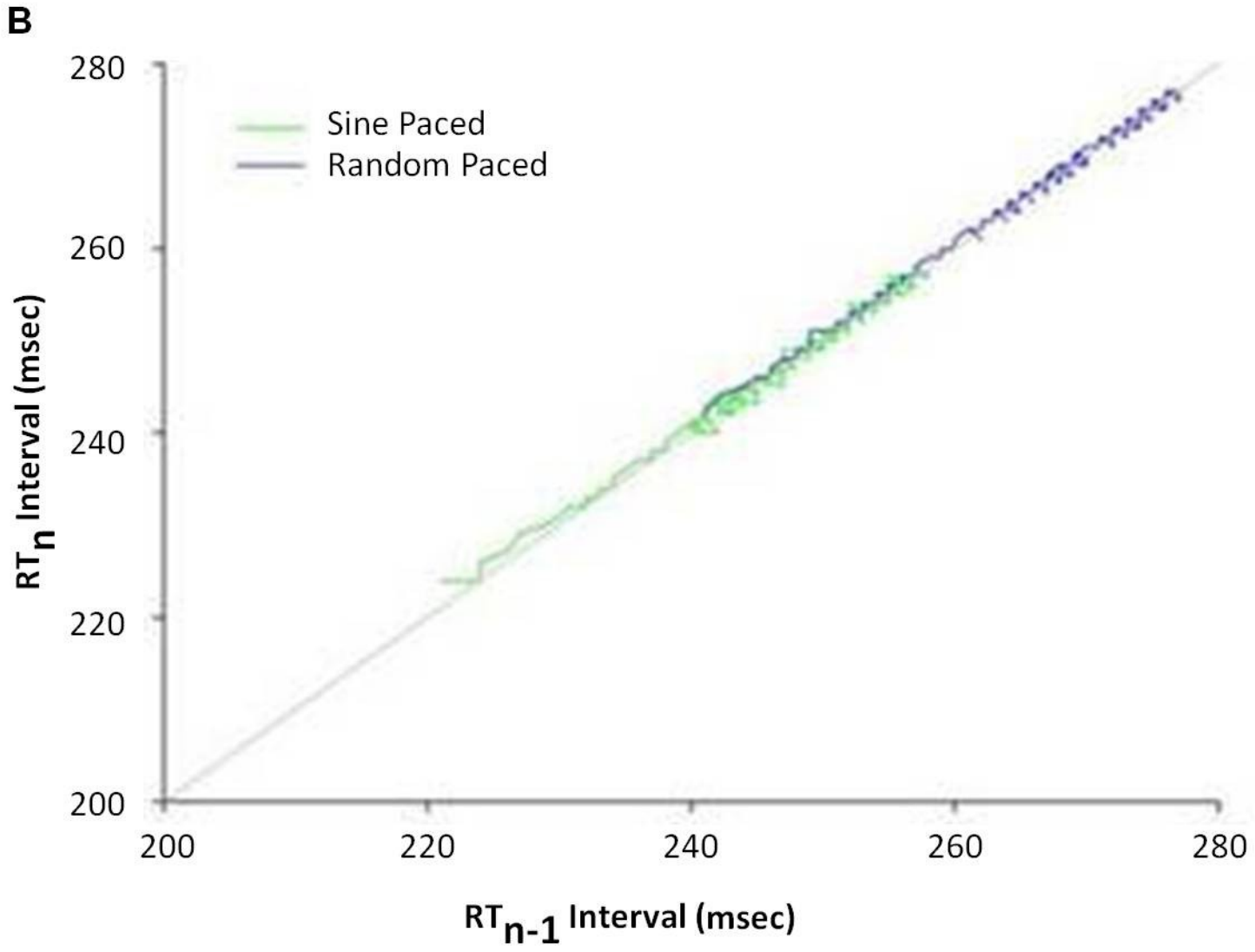


Figure B.3 - Figure 3.6 Graph C Enlarged

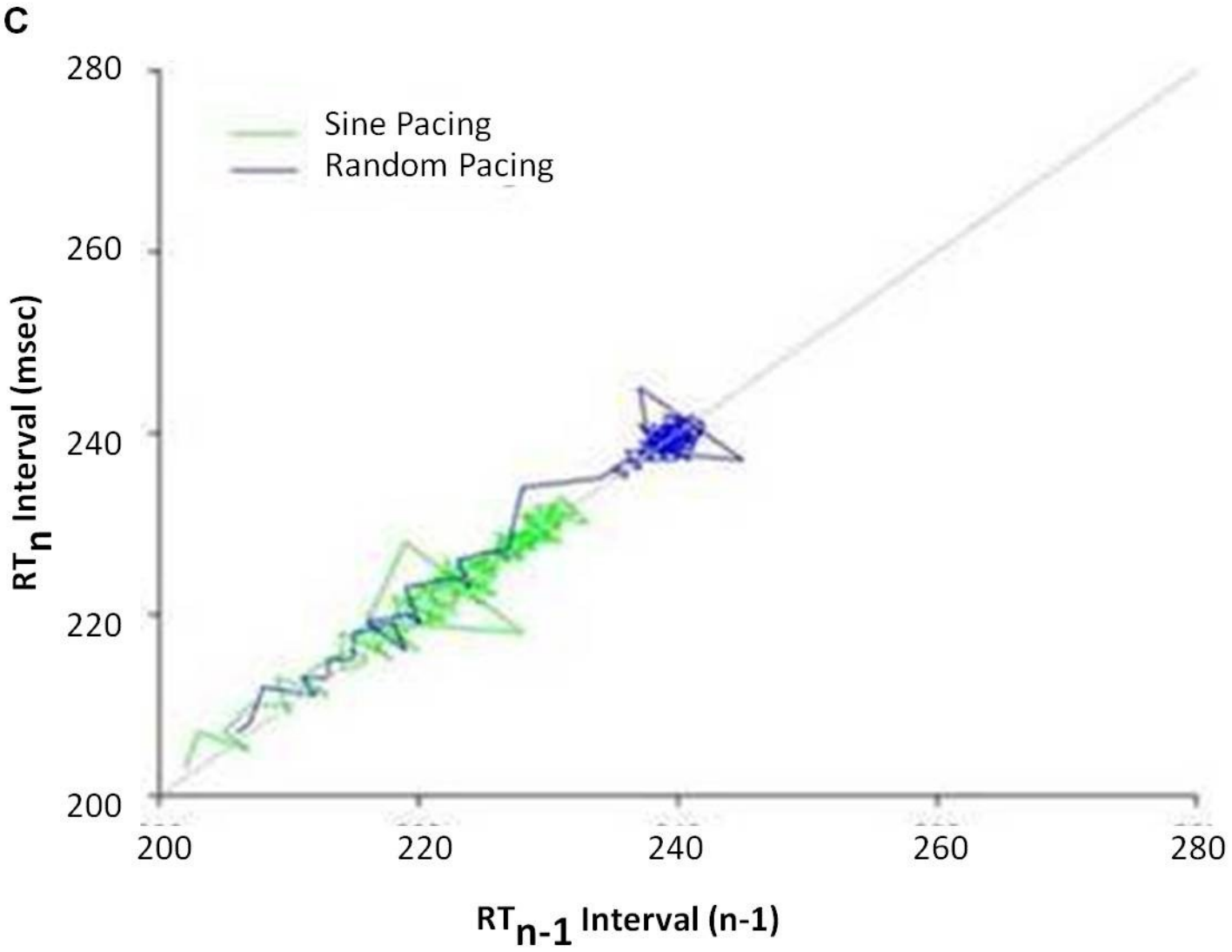


Figure B.4 - Figure 3.6 Graph D Enlarged

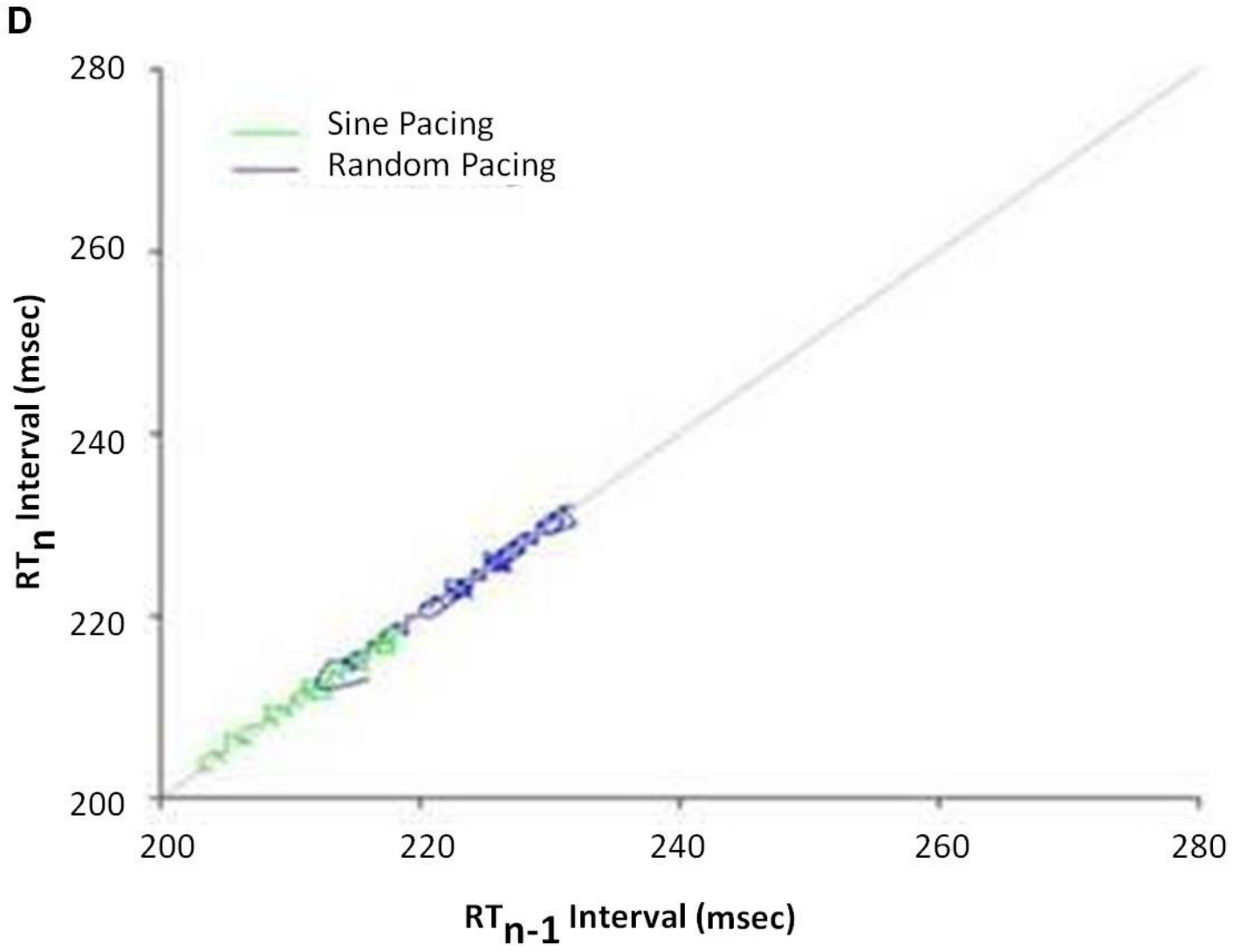


Figure B.5 - Figure 3.6 Graph E Enlarged

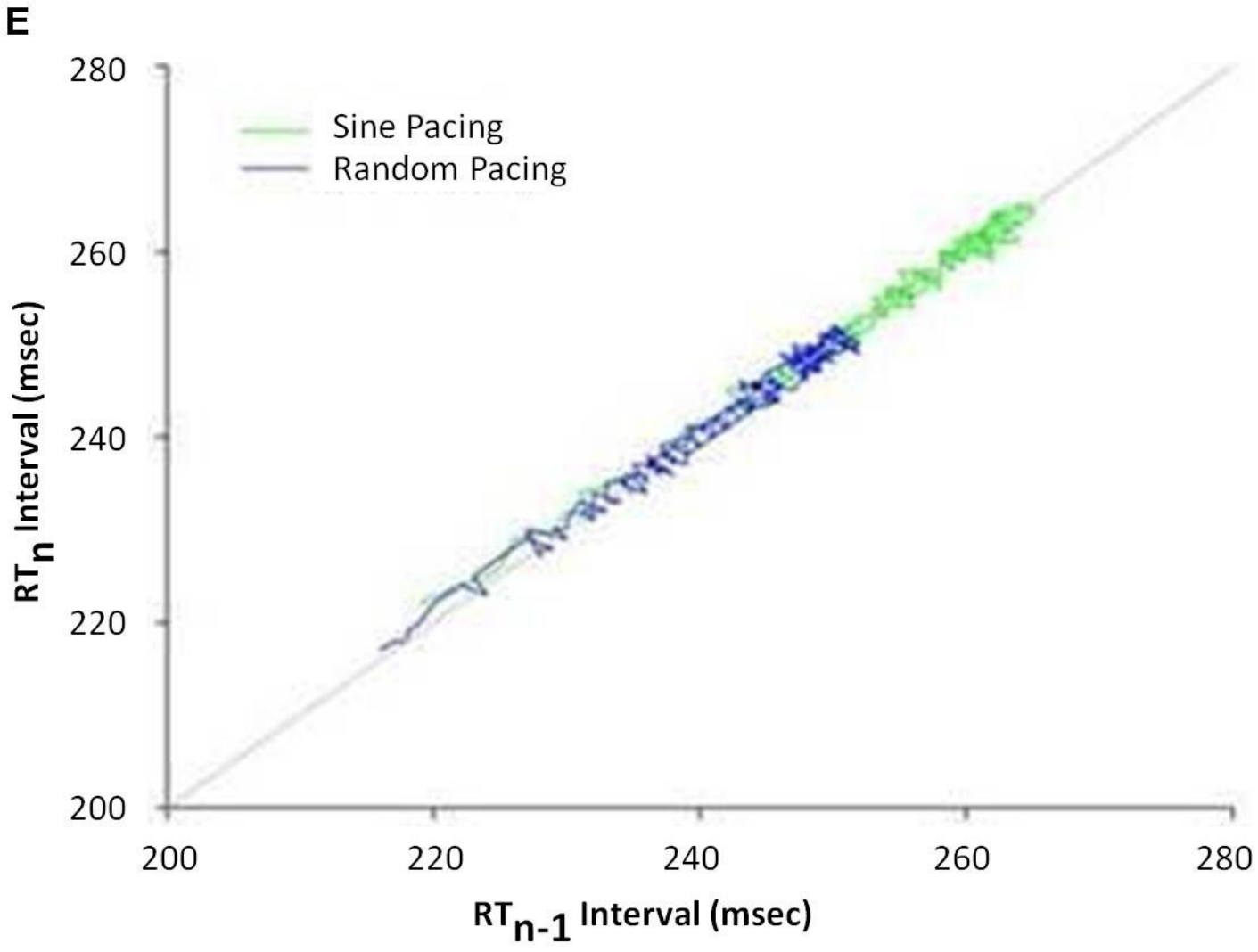


Figure B.6 - Figure 3.6 Graph F Enlarged

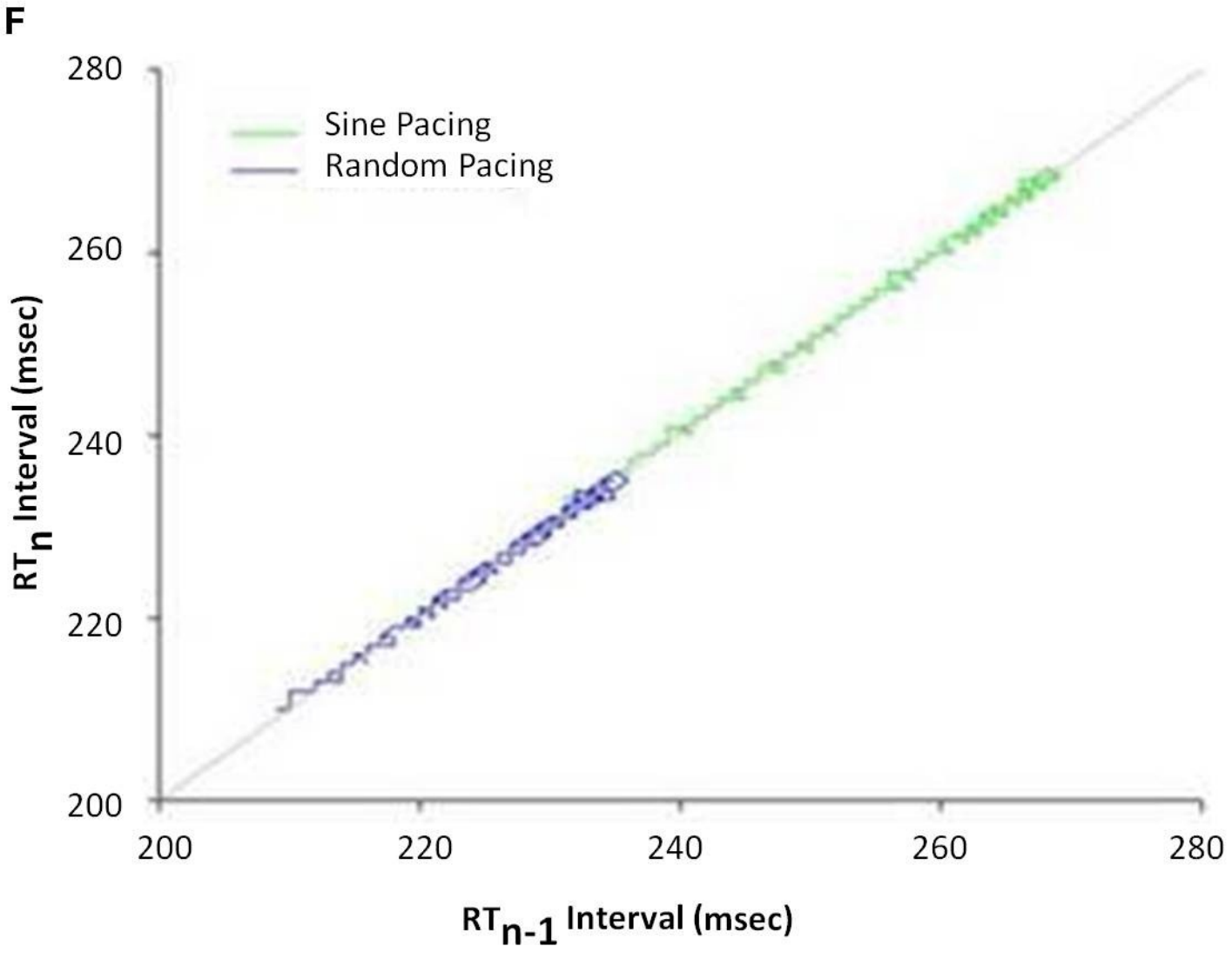
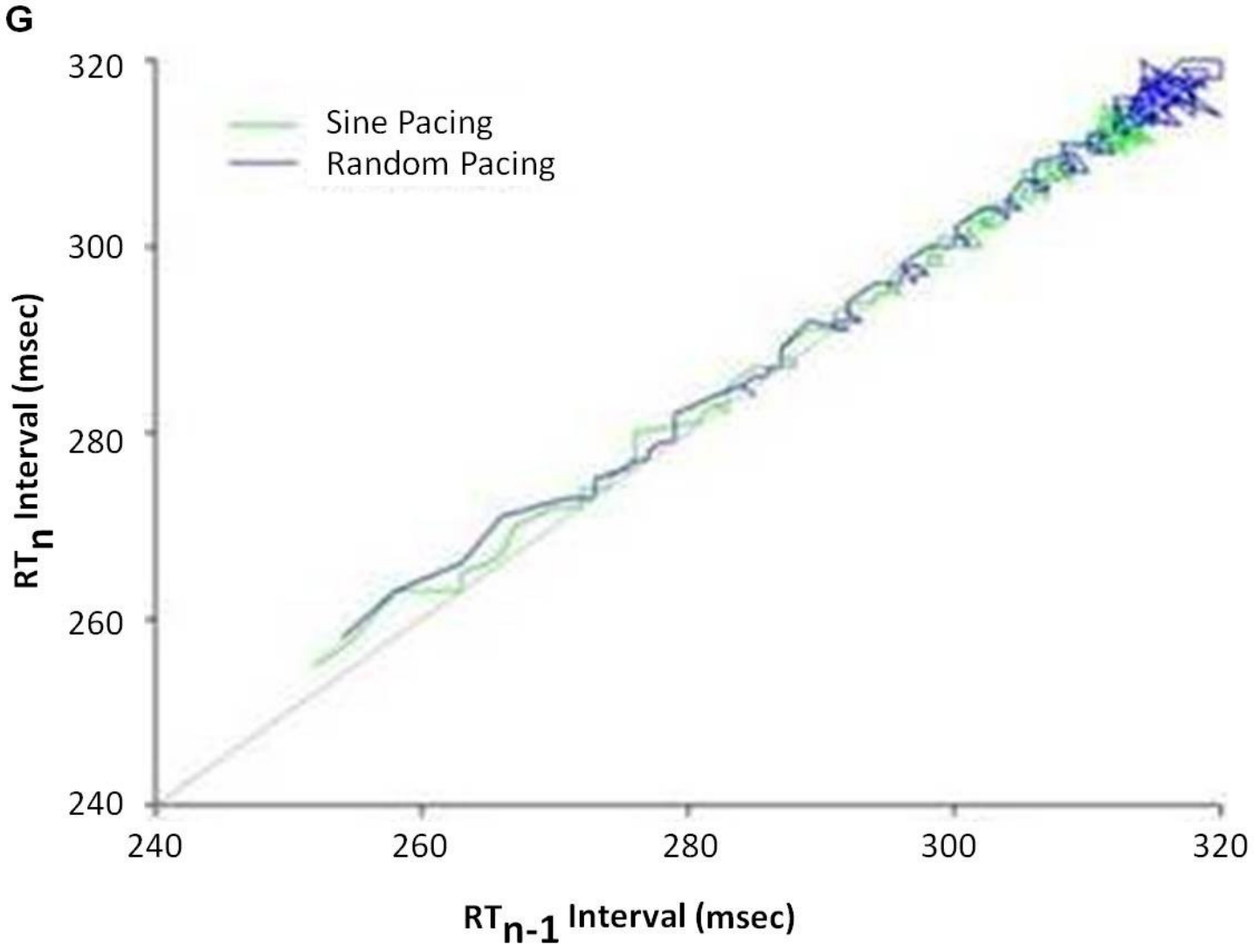


Figure B.7 - Figure 3.6 Graph G Enlarged



APPENDIX C - FIGURE 3.7

QT (RT) interval adaptations over 250 beats following the termination of fixed, sinusoidal (Sine), and random tachypacing. Each graph represents a single animal (n=7).

Figure C.1 - Figure 3.7 Graph A Enlarged

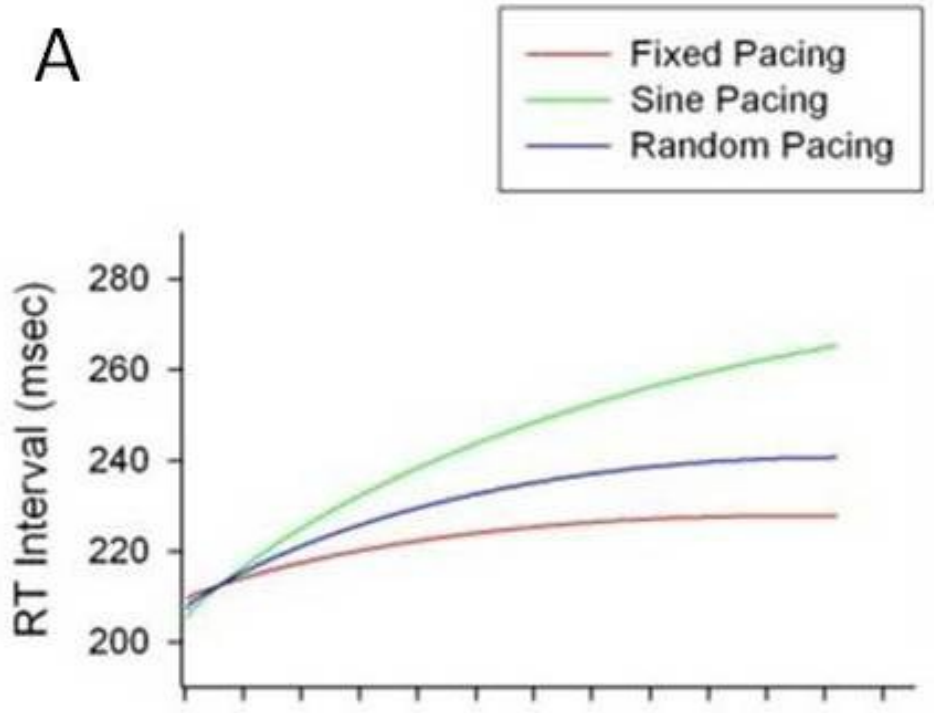


Figure C.2 - Figure 3.7 Graph B Enlarged

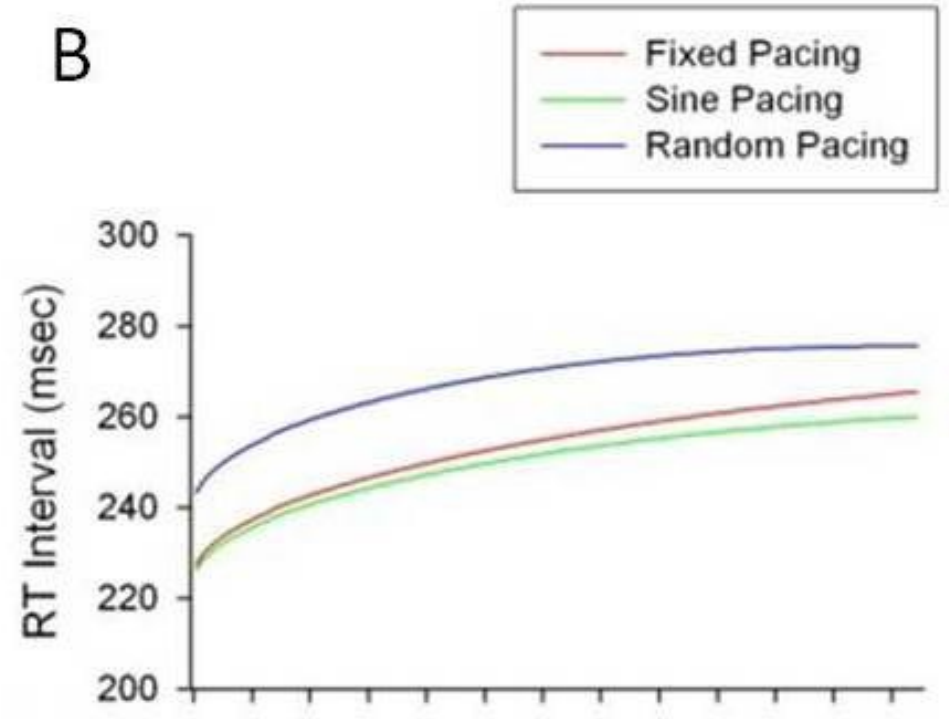


Figure C.3 - Figure 3.7 Graph C Enlarged

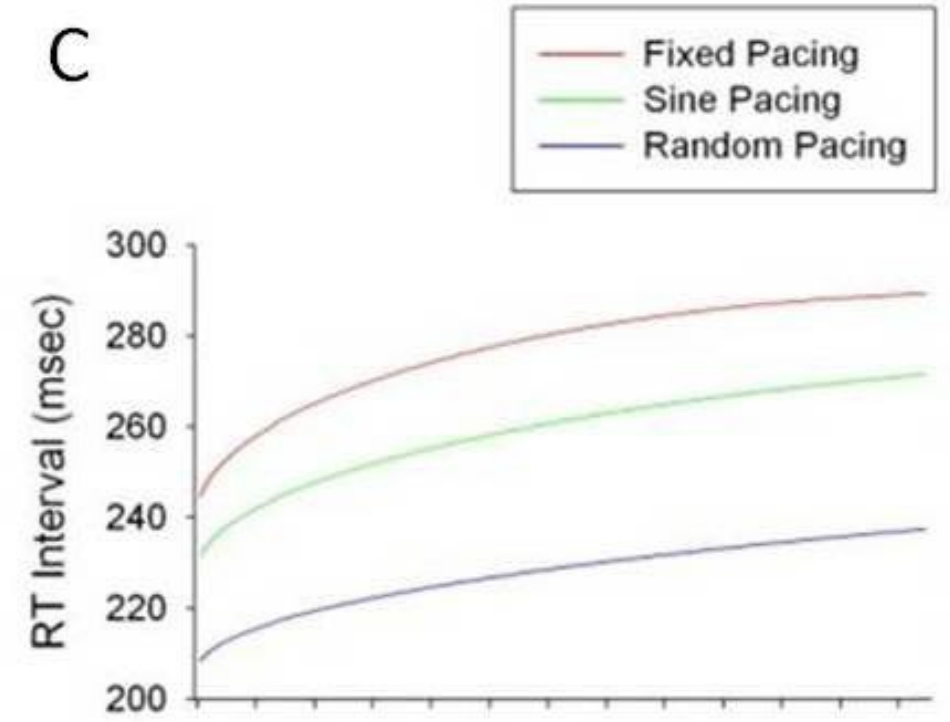


Figure C.4 - Figure 3.7 Graph D Enlarged

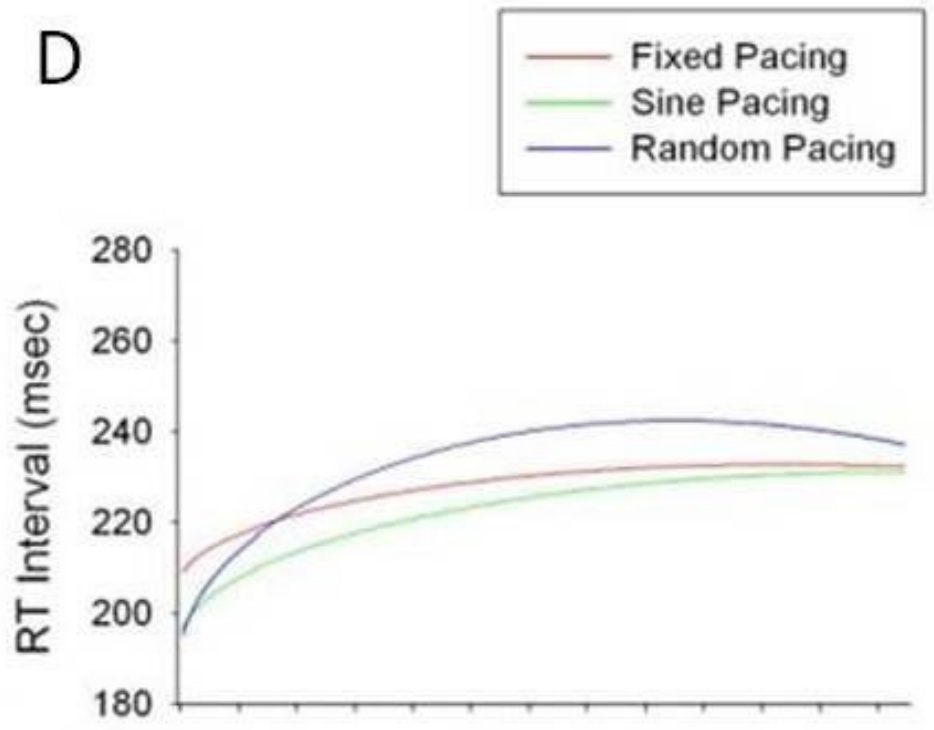


Figure C.5 - Figure 3.7 Graph E Enlarged

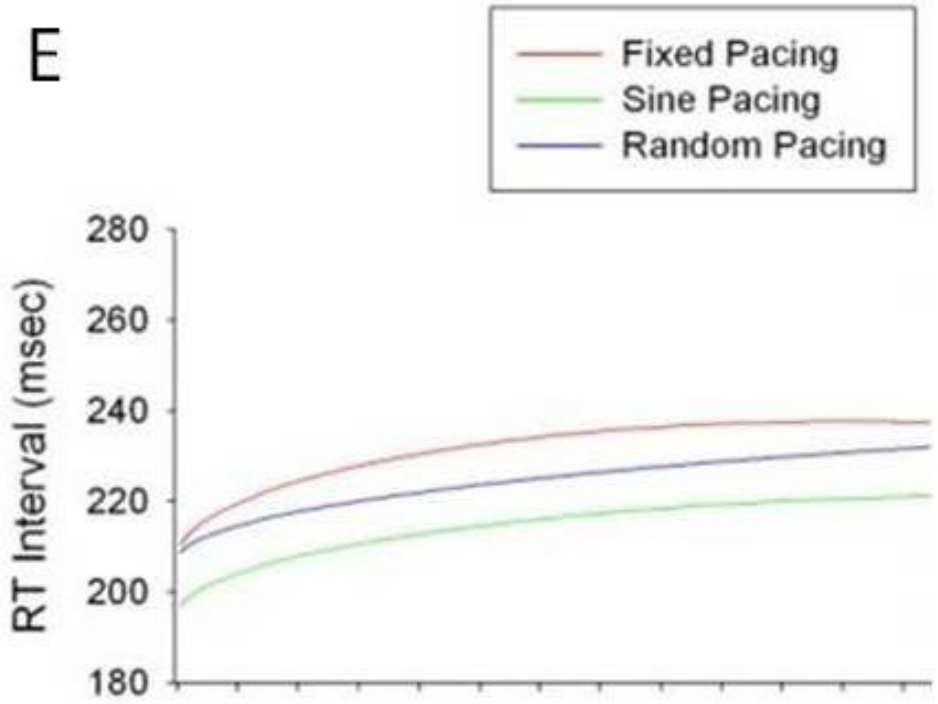


Figure C.6 - Figure 3.7 Graph F Enlarged

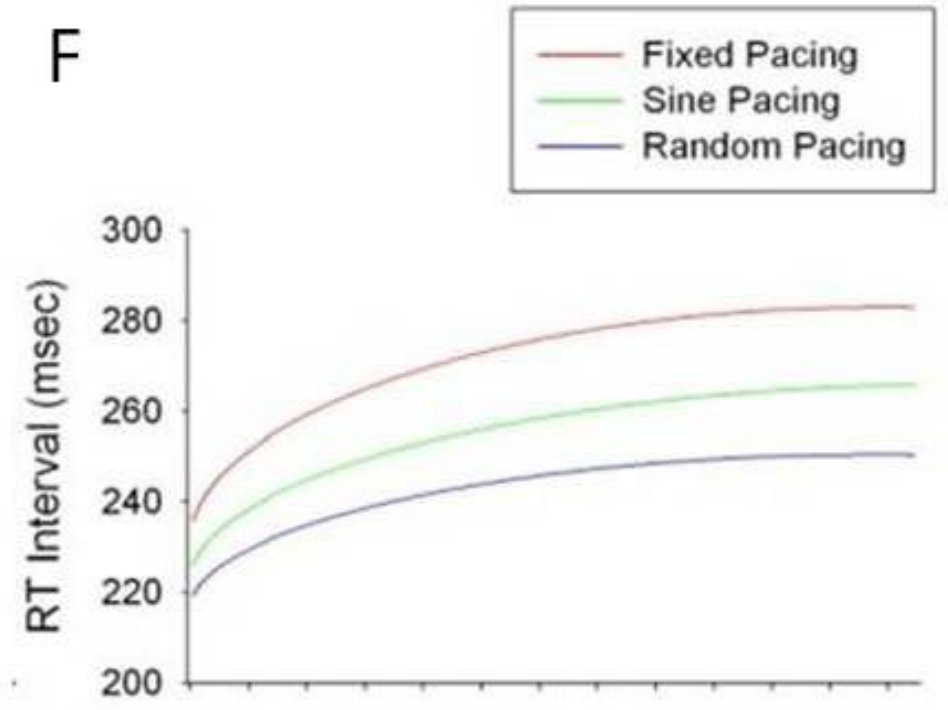
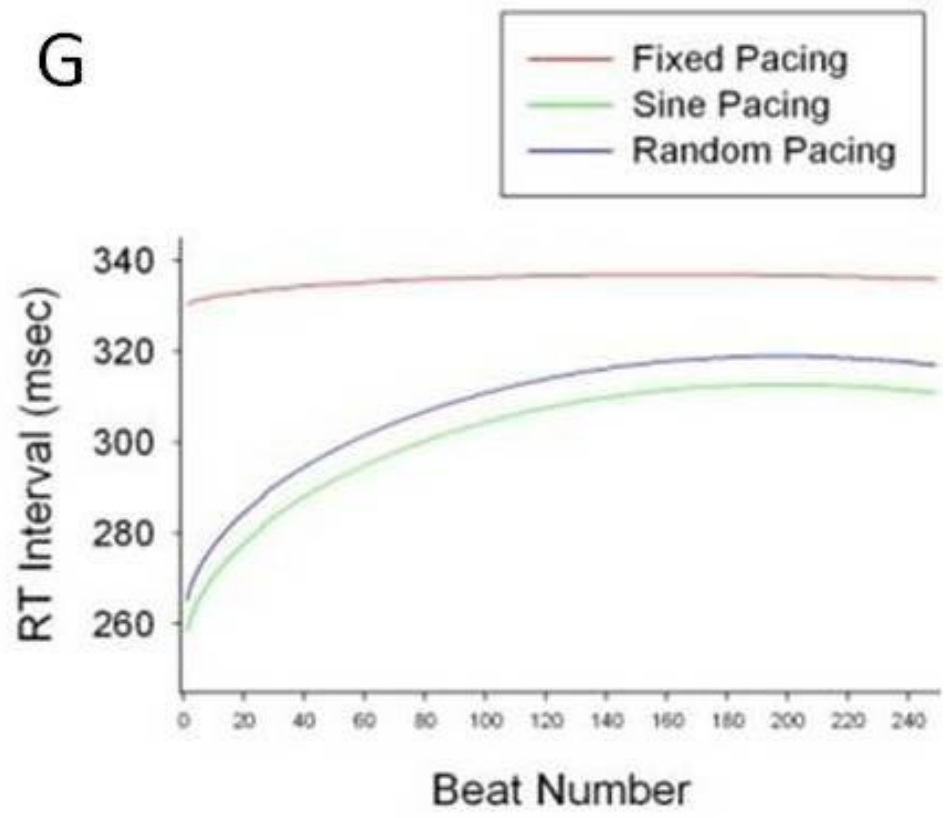


Figure C.7 - Figure 3.7 Graph G Enlarged



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